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(54) Title: GBS TOXIN RECEPTOR		
(57) Abstract A novel GBS toxin receptor, and methods for its preparation and use are provided. GBS toxin receptor polynucleotides and polypeptides are provided as well as detection, screening, and therapeutic methods and pharmaceutical compositions involving such polynucleotides and polypeptides.		

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GBS TOXIN RECEPTOR

INTRODUCTIONTechnical Field

5 This invention provides compositions and methods relating to GBS toxin receptor polynucleotides and polypeptides. The invention relates to a receptor for a polysaccharide isolated from a bacterial source.

Background

 Group B β -hemolytic Streptococci (GBS) are ubiquitous microorganisms.
10 GBS is not known to cause any harmful infections in humans except for very young babies. GBS pneumonia, also called "early-onset disease", is associated with high morbidity and mortality in newborn infants.

 In a series of studies conducted by Dr. Carl G. Hellerqvist and his associates at the Vanderbilt University School of Medicine, Nashville, Tennessee, a polysaccharide
15 GBS toxin was identified. This toxin was determined to be a major factor in the complications of GBS pneumonia, and was found to be useful as a therapeutic agent in combating tumors though inhibition of vascularization (U.S. Patent No. 5,010,062).

 In addition, as described in U.S. Patent No. 5,858,991 and WO98/32453, GBS toxin facilitates wound healing in patients by minimizing scarring and accelerating
20 healing, and reduces wound-related tumor progression.

 WO98/32452 and WO98/32448 describe the use of GBS toxin as a therapeutic agent for treating patients with chronic inflammatory diseases, such as rheumatoid arthritis and psoriasis, and for enhancing repair of neural injury.

 Prior to this invention, receptors for GBS toxin had not been identified. The
25 inventors, believing receptors of GBS toxin to reside on cells in the developing vasculature of tissues undergoing angiogenesis in the conditions described above, embarked upon a series of experiments resulting in the present invention.

SUMMARY OF THE INVENTION

For the first time, novel receptors for group B β -hemolytic Streptococcus GBS toxin (GBS toxin receptor) have been identified. One aspect of the invention provides a polypeptide comprising a GBS toxin receptor or polypeptide fragment thereof.

- 5 Preferred embodiments include mammalian GBS toxin receptors. Also provided is an antibody that recognizes GBS toxin receptor or a fragment thereof. The polypeptide of the invention can be used, *inter alia*, for the screening of compounds that can be used to treat or prevent conditions arising from pathologic or hypoxia-driven angiogenesis or neovascularization, such as, for example, cancerous tumors, chronic
10 inflammatory disease, scarring during wound healing, keloids, neural injury, and reperfusion injury.

- Another aspect of the invention provides a polynucleotide encoding a GBS toxin receptor or a fragment thereof and a polynucleotide hybridizable to such polynucleotide. Preferred polynucleotides are at least 10 bases in length and comprise
15 a nucleic acid sequence encoding, or are complementary to a nucleic acid sequence encoding, a mammalian GBS toxin receptor or a polypeptide fragment thereof.

- A third aspect of the invention is a complex comprising a GBS toxin bound to a mammalian toxin receptor or fragment thereof. Also provided is a method of forming such complex. The method comprises contacting a GBS toxin with a
20 polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide, and allowing the complex to form.

- Yet another aspect of the invention is a method for purifying a compound that binds a GBS toxin receptor. The method comprises providing a polypeptide
25 comprising a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, contacting the polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide, and separating the bound compound from the remainder of the sample.

- Another aspect of the invention is a method of determining the presence or
30 absence of GBS toxin in a sample. The method comprises contacting the sample with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, under conditions that allow specific binding of GBS toxin to the GBS toxin receptor, and determining whether specific binding of GBS toxin has

occurred. Presence of GBS toxin in a sample obtained from a neonate is indicative of early onset disease.

A sixth aspect of the invention is a method for detecting pathologic vasculature in a mammalian tissue. The method comprises detecting the presence of a GBS toxin receptor. The method can be used for detecting or monitoring a variety of medical conditions associated with angiogenesis or neovascularization, such as, for example, detecting metastasis of a cancerous tumor, or monitoring the margin of a tumor in a mammal undergoing a therapy for cancer.

Another aspect of the invention provides methods for the identification of drug candidates for the treatment of medical conditions characterized by pathologic and/or hypoxia-driven angiogenesis or neovascularization. One embodiment is a method for identifying a compound that specifically binds a mammalian GBS toxin receptor. The method comprises combining a test compound with a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that allow specific binding to occur, and detecting a complex formed between the test compound and the polypeptide. Another embodiment is a method for determining cytotoxicity of a test chimeric compound. The method comprises exposing a cell expressing a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, to a test chimeric compound comprising a cytotoxic agent coupled to GBS toxin, and detecting signs of toxicity. Yet another embodiment is a method for identifying an inhibitor of a GBS toxin receptor by incubating test cells that express GBS toxin receptor, or a fragment thereof, in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, and comparing the proliferation or migration of the test cells incubated in the presence and absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor. An inhibitor of endothelial cell proliferation or migration can be identified by the above method, wherein less proliferation or migration of test cells in the presence of the test compound is indicative of the test compound being an inhibitor of endothelial cell proliferation or migration. A therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic angiogenesis or neovascularization can also be identified by the above method, wherein less proliferation or migration of test cells in the presence of the test compound is indicative of the test compound being a candidate

therapeutic compound for the treatment or prevention of the medical condition.

The invention also provides a method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor. The method comprises simulating and selecting the most probable conformations of a mammalian GBS toxin receptor, designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the polypeptide, chemically synthesizing the analog, and evaluating the bioactivity of the analog. Also provided is a method for identifying a compound which binds to a mammalian GBS toxin receptor. The method comprises simulating and selecting the most probable conformations of a mammalian GBS toxin receptor, deducing the most probable binding domains of the polypeptide, designing a compound that would form the energetically most probable complexes with the polypeptide, chemically synthesizing the compound, and evaluating the bioactivity of the compound.

Another aspect of the invention is a method for the prevention or treatment of neonatal onset disease in a human neonate by administering an inhibitor of binding of GBS toxin to a human GBS toxin receptor.

Yet another aspect of the invention is a method for inhibiting pathologic or hypoxia-driven endothelial cell proliferation or migration in a mammalian tissue. The method comprises specifically binding a molecule to a GBS toxin receptor present on the surface of at least one cell in the tissue, the molecule being selected from the group consisting of a compound that can evoke an inflammatory response when bound to a GBS toxin receptor in a mammal, a chimeric compound comprising a cytotoxic compound coupled to a compound that specifically binds the GBS toxin receptor, an inhibitor of GBS toxin receptor phosphorylation, and an inhibitor of GBS toxin receptor activity.

The invention also provides a GBS toxin receptor or fragment thereof, an inhibitor of a GBS toxin receptor, or an inhibitor of binding of a GBS toxin to a GBS toxin receptor, for use in a method of treatment of the human or animal body or for the manufacture of a medicament for the treatment of a medical condition characterized by pathologic or hypoxia-driven angiogenesis or neovascularization. Also provided is a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor for use in a method of treatment of the human or animal body.

Also provided are pharmaceutical compositions comprising an inhibitor of a

GBS toxin receptor and/or a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor, and a pharmaceutically acceptable carrier.

- The invention also provides kits comprising a GBS toxin receptor or fragment and/or reagents for detecting the presence of a GBS toxin receptor or polypeptide fragment thereof or the presence of a polynucleotide encoding same.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a process of rational drug design.

- FIGS. 2A and 2B depict the results of immunohistochemical analysis of GBS toxin receptor expression in cancerous and normal human ovary tissue, respectively, using antibody Pab55 as described in Example 4.

- FIGS. 3A and 3B depict the results of immunohistochemical analysis of GBS toxin receptor expression in cancerous and normal human ovary tissue, respectively, using antibody Pab57 as described in Example 4.

FIGS. 4A-4C depict the targeted delivery of a chimeric compound to GBS toxin receptor expressed in a cancerous tissue as described in Example 6.

DESCRIPTION OF SPECIFIC EMBODIMENTS

DEFINITIONS

- Generally, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, and microbial culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification steps supplied by manufacturers are typically performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references (See generally, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring

Harbor, N.Y.) which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical chemistry, organic synthetic chemistry, and pharmaceutical formulation described below are those well known and commonly employed in the art. Standard techniques can be used for chemical
5 syntheses, chemical analyses, pharmaceutical formulation and delivery, and treatment of patients. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

By "GBS toxin receptor" is meant a proteinaceous molecule capable of binding a toxin from Group B β -hemolytic *Streptococcus* bacteria (GBS toxin), such
10 as, for example, CM101. A GBS toxin receptor is usually found in nature on the surface of a cell. Recombinant membrane bound and soluble GBS toxin receptors can be produced by laboratory techniques known in the art and described herein.

The term "isolated polynucleotide" referred to herein means a polynucleotide that has been subjected to manipulation, such that the isolated polynucleotide is no
15 longer associated with the chromosome or cell that the polynucleotide is normally associated with in nature in the same manner as it is normally associated in nature. An example of an "isolated polynucleotide" is a polynucleotide of genomic, recombinant, or synthetic origin or some combination thereof.

The term "isolated protein" referred to herein means a protein that is no longer
20 associated with the cell that the protein is normally associated with in nature in the same manner as it is normally associated in nature, such as (1) a protein free of at least some other proteins from the same source, (2) a protein expressed by a cell from a different species, (3) a protein that does not occur in nature, and (4) a protein produced from cDNA, recombinant RNA, or synthetic origin or some combination
25 thereof.

The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus.

The term "naturally occurring" means found in nature. For example, a
30 polypeptide or polynucleotide sequence that is present in an organism (including viruses) found in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "operably linked" refers to a juxtaposition wherein the components

so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

5 The term "control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control
10 sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is necessary for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

15 The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. The term includes single- and double-stranded forms of DNA.

 The term "oligonucleotide" referred to herein includes naturally occurring, and
20 modified nucleotides linked together by naturally occurring and non-naturally occurring oligonucleotide linkages. An oligonucleotide is usually a polynucleotide 200 bases or fewer in length. Preferably oligonucleotides are minimally 10 to 60 bases in length and most preferably
25 15-35 bases in minimal length. Oligonucleotides are usually single-stranded, e.g. for probes; although oligonucleotides may be double-stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides. The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified
30 nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. An oligonucleotide can include a label for detection, if desired.

By "complementary" or "complement" is meant that wherever adenine appears in a first nucleic acid sequence, thymine or uracil is found in the "complementary" sequence and vice versa, and wherever guanine appears in a first nucleic acid sequence, cytosine is found in the "complementary" sequence and vice versa.

The term "sequence identity" describes the proportion of base matches between two nucleic acid sequences or the proportion of amino acid matches between two amino acid sequences, i.e. the degree of identity between two sequences. When sequence identity is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of exact matches over the length of sequence from a GBS toxin receptor sequence that is compared to some other sequence. Various computer alignment programs can be used to determine sequence identity. In its simplest form, % identity is calculated by dividing the number of exact matches between two nucleic acid sequences or between two amino acid sequences by the total number of nucleotides or amino acids in the reference sequence. For example, if there are 300 matches between sequences 400 amino acids in length, the sequences have 75% identity. Uracil and thymine are considered identical when comparing a ribonucleic acid sequence with a deoxyribonucleic acid sequence.

As applied to polynucleotides, the term "substantial identity" means that two nucleic acid sequences when optimally aligned, such as by the program BLAST (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990)), share at least about 85%, preferably at least about 90% sequence identity and most preferably 95% or greater sequence identity. When using computer alignment programs, gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less are usually used; 6 bases or less are preferred; 2 bases or less are most preferred. When using oligonucleotides as probes or in treatments, the sequence identity between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and most preferably not less than 19 matches out of 20 possible base pair matches (95%).

Preferably, bases which are not identical nevertheless are part of a degenerate codon that encodes the same amino acid at that amino acid position. Alternatively, bases which are not identical preferably are part of a degenerate codon that encodes a conservative amino acid substitution for that amino acid position.

5 As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned by the BLAST computer program, share at least about 80 percent sequence identity, preferably at least about 86 percent sequence identity, more preferably at least about 95 percent sequence identity, even more preferably at least about 99 percent sequence identity up to having one amino acid
10 difference, and most preferably share 100% identity. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side
15 chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino
20 acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

25 The term "hybridizable under high stringency conditions" referred to herein means capable of specific binding under conditions whereby only nucleic acid sequences having a substantial identity of greater than 95% with respect to each other will hybridize. These conditions are known in the art and discussed herein.

The term "degenerate codon" means any of the nucleotide codon triplets
30 encoding a desired amino acid according to the genetic code. Codons can be selected based upon known preferred codon usage in a host organism such as *E. coli*.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-

occurring sequence deduced, for example, from a full-length DNA sequence.

Fragments typically are at least 3 amino acids long, preferably are 5-10 amino acids long, more preferably are 10-50 amino acids long, even more preferably are more than 50 amino acids long and comprise at least one extracellular domain of a GBS toxin receptor. Most preferred are fragments that comprise the entire extracellular domains of a GBS toxin receptor, and preferably also comprise portions of transmembrane and intracellular domains sufficient to maintain the polypeptide fragment in a functional stereochemical conformation on the surface of a cell, lipid membrane, liposome, micelle, or other lipophilic structure.

5 The term "immunologically reactive" means having antigenic properties or being capable of being specifically bound by an antibody that can specifically bind GBS toxin receptor. A substance has antigenic properties if it can generate monoclonal or polyclonal antibodies when administered to an animal under conditions known in the art to facilitate the production of antibodies that will recognize and bind
15 a particular antigen.

A "heterologous polypeptide" is a polypeptide different from polypeptides normally produced by a particular cell. For example, a GBS toxin receptor polypeptide or fragment thereof that is produced recombinantly in a cell that does not normally produce such GBS toxin receptor polypeptide or fragment thereof, is a
20 heterologous polypeptide. A second polypeptide joined to a GBS toxin receptor polypeptide or fragment thereof is also a heterologous polypeptide if it is not joined to a GBS toxin receptor polypeptide in nature.

As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to
25 a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes (e.g., ^3H , ^{14}C ,
30 ^{35}S , ^{125}I , ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some

embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The term "compound" as used herein preferably refers to a peptidic, peptidomimetic, organic, or other chemical molecule and also refers to a nucleic acid molecule or chemical derivative thereof. The compound can interact with, or be, the polynucleotides or polypeptides of the invention.

The singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

The SEQ ID NOs of the nucleic acid and amino acid sequences described herein are summarized below in Table 1.

Table 1
Nucleic Acid and Amino Acid Sequences

SEQ ID NO:	Type of Sequence	Description
SEQ ID NO: 1	nucleic acid	Partial human GBS toxin receptor (HP55)
SEQ ID NO: 2	amino acid	Partial human GBS toxin receptor (HP55)
SEQ ID NO: 3	nucleic acid	Sheep GBS toxin receptor (SP55)
SEQ ID NO: 4	amino acid	Sheep GBS toxin receptor (SP55)
SEQ ID NO: 5	nucleic acid	Primer
SEQ ID NO: 6	nucleic acid	Primer
SEQ ID NO: 7	nucleic acid	Full-length human GBS toxin receptor (HP59)
SEQ ID NO: 8	amino acid	Full-length human GBS toxin receptor (HP59)
SEQ ID NO: 9	nucleic acid	Human/Sheep consensus GBS toxin receptor coding region (with base codes a, c, g, t, m, r, w, s, y, k)
SEQ ID NO: 10	amino acid	Human/Sheep consensus GBS toxin receptor coding region (translation of SEQ ID No: 9)
SEQ ID NO: 11	nucleic acid	Human/Sheep consensus GBS toxin receptor coding region (with base codes a, c, g, t, n)
SEQ ID NO: 12	amino acid	Human/sheep consensus GBS toxin receptor coding region (translation of SEQ ID NO: 11)

The headings provided herein describe the general topic discussed and are not intended to be exclusive of information discussed in other sections. Frequently, information, methods, compositions, and other aspects may be applicable to more than one embodiment of the invention and can be so combined.

INTRODUCTION

GBS toxin binds to tissues undergoing pathologic, hypoxia-driven, and

embryologic angiogenesis or neovascularization. The inventors have identified at least two mammalian GBS toxin receptors, which are described herein. Examples 1 and 2 describe the cloning and characterization of some GBS toxin receptors. The inventors have classified GBS toxin receptor as an integral protein with seven transmembrane domains. The predicted segments are shown in Table 7. The protein has several putative sites for phosphorylation by cAMP-dependent kinase, protein kinase C (PKC), and casein kinase II (CK2). Typically, such integral proteins, upon binding of a molecule (e.g., a ligand or an extracellular messenger), undergo a conformational change which facilitates phosphorylation at phosphorylation sites such as those discussed above. The phosphorylation of the protein at these sites may trigger a signal transduction cascade, which often results in proliferation or other nuclear responses of the cells which have been exposed to the binding molecule. Angiogenesis or neovascularization involves proliferation and migration of endothelial cells. As discussed in greater detail in Examples 4 and 5, GBS toxin receptor expression is correlated with medical conditions involving pathologic, hypoxia-driven, and embryogenic angiogenesis or neovascularization. GBS toxin receptor polypeptides can be used for a variety of purposes, including screening for compounds that can inhibit endothelial cell proliferation and/or migration mediated by GBS toxin receptor and screening for cytotoxic chimeric compounds that can bind to and destroy cells expressing GBS toxin receptor. GBS toxin receptor polynucleotides can be used for a variety of purposes, including the design of antisense polynucleotides that can block translation of messenger RNA encoding GBS toxin receptor.

25 POLYNUCLEOTIDES

One aspect of the invention provides for isolated polynucleotides at least ten bases in length encoding or complementary to a nucleic acid sequence encoding a GBS toxin receptor or a fragment derived therefrom. Preferably, the GBS toxin receptor is a mammalian GBS toxin receptor, more preferably an ovine, bovine or feline GBS toxin receptor, and most preferably a human GBS toxin receptor. The isolated polynucleotides can be naturally occurring or non-naturally occurring. The isolated polynucleotides can comprise a DNA sequence or an RNA sequence in which every T is replaced with U. For purposes of determining percentage identity, T is considered equivalent to U. Preferably, the polynucleotides include alleles of an

ovine, bovine, feline or human GBS toxin receptor, and can include alleles of GBS toxin receptor of other mammals. These polynucleotides can be isolated using polynucleotides derived from SEQ ID NOs: 1, 3, 7, 9 and 11, as described further below.

- 5 Polynucleotides, oligonucleotides and fragments of the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. The polynucleotides can be hybridizable under high stringency conditions to a nucleic acid molecule having a nucleic acid sequence comprising at least 20 contiguous
- 10 polynucleotides, preferably at least 30 contiguous nucleotides of SEQ ID NO: 1 or SEQ ID NO: 3, and even more preferably to the nucleic acid sequence of SEQ ID NO: 1, 3, 7, 9 or 11 or the complement of SEQ ID NO: 1, 3, 7, 9 or 11. Such polynucleotides can be used for performing selective, high stringency hybridization and are particularly useful for performing amplification of nucleic acid by polymerase
- 15 chain reaction (PCR) to determine the presence or absence of GBS toxin receptor in a sample, for isolating a naturally occurring nucleic acid encoding a GBS toxin receptor (see Example 3), as antisense molecules for blocking translation of GBS toxin receptor mRNA. Particularly preferred are polynucleotides hybridizable under high stringency conditions to a nucleic acid molecule having a nucleic acid sequence
- 20 comprising the nucleic acid sequence of nucleotides 266 to 1870 of SEQ ID NO: 7 (the putative full length coding region of a human GBS toxin receptor, excluding the start codon), nucleotides 266 to 1870 of SEQ ID NO: 7 (the putative full length coding region of a human GBS toxin receptor, including the start codon), nucleotides 61 to 1542 of SEQ ID NO: 1 (the partial coding region of a human GBS toxin receptor,
- 25 excluding the start codon), nucleotides 58 to 1542 of SEQ ID NO: 1 (the partial coding region of a human GBS toxin receptor, including the start codon), nucleotides 87 to 1568 of SEQ ID NO: 3 (the coding region of a sheep GBS toxin receptor, excluding the start codon), nucleotides 84 to 1568 of SEQ ID NO: 3 (the coding region of a sheep GBS toxin receptor, including the start codon), or a complementary nucleic acid sequence thereof.
- 30

The polynucleotides can have an identity to the nucleic acid sequence of a corresponding region of SEQ ID NO: 1, 3 or 7 or the complement of a corresponding region of SEQ ID NO: 1, 3 or 7 in the range of about 85% to 100%, preferably greater than about 87% identity, more preferably greater than about 95% identity, and most

preferably about 99% to 100% identity. Particularly preferred are polynucleotides comprising the nucleic acid sequence of nucleotides 266 to 1870 of SEQ ID NO: 7, or nucleotides 87 to 1568 of SEQ ID NO: 3, SEQ ID NO: 9, SEQ ID NO: 11, or a complementary nucleic acid sequence thereof.

- 5 Preferably, the polynucleotides comprise a nucleic acid sequence encoding, or complementary to a nucleic acid sequence encoding, a polypeptide having an identity to the amino acid sequence of a fragment of a GBS toxin receptor in the range of about 85% to 100%, more preferably greater than 86% identity, even more preferably greater than 95% identity, and most preferably 99% to 100% identity. Preferably, the
- 10 fragment binds GBS toxin. Preferred fragments comprise all or a portion of residues 1 to 495 of SEQ ID NO: 2 or all or a portion of residues 1 to 536 of SEQ ID NO: 8. Particularly preferred are polynucleotides comprising a nucleic acid sequence encoding a polypeptide having 100% identity to the amino acid sequence of residues 1 to 495 of SEQ ID NO: 4, residues 1 to 495 of SEQ ID NO: 2, or residues 1 to 536 of
- 15 SEQ ID NO: 8.

- Polynucleotides encoding naturally occurring GBS toxin receptor can be isolated from various tissue sources and cell cultures from different species that produce such a receptor by the methods described herein, such as, for example, cells from tumor endothelium, synovial tissue in rheumatoid arthritis, or hypoxic tissue
- 20 deprived of or restricted from blood flow, such as in reperfusion injury or wounded tissue. Such polynucleotides can be isolated by hybridization using probes or by polymerase chain reaction using oligonucleotides, as well as by implementing other molecular biology techniques known in the art. Such probes and oligonucleotides typically comprise various regions of the sequence of SEQ ID NO: 1, 3, 7, 9 or 11,
- 25 preferably of SEQ ID NO: 1, 3, or 7, or encode various regions of the sequence of SEQ ID NO: 2, 4, 8, 10 or 12, preferably of SEQ NO: 2, 4 or 8.

- Polynucleotides useful for cloning genes encoding GBS toxin receptors of various organisms can be determined by comparing the amino acid sequences of homologous proteins. (see Table 4). For example, conserved regions can be targeted
- 30 for the synthesis of oligonucleotides or degenerate oligonucleotides to be used as probes for hybridization or nucleic acid amplification, techniques discussed further below and in Example 3. Stringency can be varied to achieve selective hybridization conditions whereby nucleic acid sequences having less than 95% identity with respect to each other will hybridize. These conditions are known in the art and discussed

herein and examples are provided. Generally, the nucleic acid sequence identity between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least about 85%, and more typically with preferably increasing identities of at least about 90%, 95%, 99%, and 100%.

- 5 Polynucleotides can be used as probes under high stringency wash conditions and with corresponding hybridization conditions, as known in the art. Small polynucleotides, for example, polynucleotides 200 bases or fewer in length, are often referred to in the art as oligonucleotides. Techniques for using polynucleotides as probes to detect the same or related nucleic acid sequences is well known in the art.
- 10 See, for example, Sambrook et al, especially Chapter 11, the text of which is herein incorporated by reference. Usually, probes can be made from polynucleotides that are 10 to 200 bases in length. Preferably probes are made from polynucleotides 10 to 60 nucleotides in length and most preferably 12 to 40 bases in length. Specific probes can be designed based on results obtained using nucleic acid homology computer
- 15 programs such as FASTA, which uses the method of Pearson and Lipman (*Proc. Natl. Acad. Sci. USA* 85:2444-2448 (1988)) and shows the degree of identity between compared sequences. The size of the probe is dependent upon the region of the gene to which it will be hybridized. The size of the probe increases as the degree of homology to undesirable nucleic acid sequences increases. A probe 10-50 nucleotides
- 20 in length can be used, preferably more than 50 nucleotides, even more preferably more than 100 nucleotides, and most preferably a probe made from the entire coding region of a GBS toxin receptor will be used. To decrease the number of false positives, preferably two probes are used to identify clones that bind to both probes under hybridization and wash conditions. Oligonucleotides can be synthesized on an
- 25 Applied BioSystems oligonucleotide synthesizer according to specifications provided by the manufacturer.

- Typically, hybridization and washing conditions are performed at according to conventional hybridization procedures. Typical hybridization conditions for screening plaque lifts (Benton and Davis (1978) *Science* 196: 180) can be: 50%
- 30 formamide, 5 x SSC (sodium chloride, sodium citrate) or SSPE (sodium chloride, sodium phosphate, EDTA), 1-5 x Denhardt's solution, 0.1-1% SDS, 100-200 μg sheared heterologous DNA or tRNA, 0-10% dextran sulfate, 1×10^5 to 1×10^7 cpm/ml of denatured probe with a specific activity of about 1×10^8 cpm/ μg , and

incubation at 42°C for about 6-36 hours. Prehybridization conditions are essentially identical except that probe is not included and incubation time is typically reduced. Washing conditions are typically 1-3 x SSC, 0.1-1% SDS, 42-70°C with change of wash solution at about 5-30 minutes. Cognate bacterial sequences, including allelic sequences, can be obtained in this manner. For high stringency hybridization conditions, various parameters can be altered to increase the stringency of hybridization, such as by increasing the temperature of incubation with the labeled probe. Preferably, for greater flexibility in experimental design, the probe can be hybridized at a lower temperature, such as, for example, room temperature and the stringency can then be modified by altering the salt concentration and temperature of the wash solutions. For high stringency a wash temperature of greater than or equal to 42°C can be used, such as, for example, 68°C, in a wash buffer having a salt concentration less than 3X SSC, such as, for example, 0.1X SSC. In some cases, TMACL can also be used, particularly for polynucleotides rich in G-C base pairs in order to decrease non-specific binding. A lower stringency wash can be used to hybridize polynucleotides with lower identities or polynucleotides that are less than 60 base pairs in length. For a low stringency wash, temperatures of less than or equal to 42° can be used in a wash buffer having a salt concentration of greater than or equal to 2X SSC.

The invention includes methods for amplification of target nucleic acids, such as the polymerase chain reaction ("PCR") technique. The PCR technique can be applied to identify related sequences in the genomes of various organisms and to detect nucleotide sequences in suspected samples, using oligonucleotide primers spaced apart from each other and based on the genetic sequence set forth herein. The primers are complementary to opposite strands of a double-stranded DNA molecule and are typically separated by from about 50 to 450 nucleotides or more (usually not more than 2000 nucleotides). This method entails preparing the specific oligonucleotide primers followed by repeated cycles of target DNA denaturation, primer binding, and extension with a DNA polymerase to obtain DNA fragments of the expected length based on the primer spacing. Extension products generated from one primer serve as additional target sequences for the other primer. The degree of amplification of a target sequence is controlled by the number of cycles that are performed and is theoretically calculated by the simple formula 2^n where n is the number of cycles. Given that the average efficiency per cycle ranges from about 65%

to 85%, 25 cycles produce from 0.3 to 4.8 million copies of the target sequence. The PCR method is described in a number of publications, including Saiki et al., *Science* (1985) 230:1350-1354; Saiki et al., *Nature* (1986) 324:163-166; and Scharf et al., *Science* (1986) 233:1076-1078. Also see U.S. Patent Nos. 4,683,194; 4,683,195; and 4,683,202, the text of each patent is herein incorporated by reference. Additional methods for PCR amplification are described in: PCR Technology: Principles and Applications for DNA Amplification ed. HA Erlich, Freeman Press, New York, NY (1992); *PCR Protocols: A Guide to Methods and Applications*, eds. Innis, Gelfand, Snisky, and White, Academic Press, San Diego, CA (1990); Mattila et al. (1991) *Nucleic Acids Res.* 19: 4967; Eckert, K.A. and Kunkel, T.A. (1991) *PCR Methods and Applications 1*: 17, and; *PCR*, eds. McPherson, Quirk, and Taylor, IRL Press, Oxford, all of which are incorporated herein by reference.

In yet another embodiment, an antisense polynucleotide can be administered to a mammal to treat or prevent a medical condition involving pathologic and/or hypoxia-driven angiogenesis. The antisense oligonucleotides of the invention can be synthesized by any of the known chemical oligonucleotide synthesis methods. Such methods are generally described, for example, in Winnacker, *From Genes to Clones: Introduction to Gene Technology*. VCH Verlagsgesellschaft mbH (H., Ibelgaufs trans. 1987). Any of the known methods of oligonucleotide synthesis can be utilized in preparing the instant antisense oligonucleotides. The antisense oligonucleotides are most advantageously prepared by utilizing any of the commercially available, automated nucleic acid synthesizers. The device utilized to prepare the oligonucleotides described herein, the Applied Biosystems 380B DNA Synthesizer, utilizes -cyanoethyl phosphoramidite chemistry. Antisense oligonucleotides hybridizable with any portion of the mRNA transcript can be prepared by the oligonucleotide synthesis methods known to those skilled in the art. While any length oligonucleotide can be utilized in the practice of the invention, sequences shorter than 12 bases may be less specific in hybridizing to the target GBS toxin receptor mRNA, and may be more easily destroyed by enzymatic digestion. Hence, oligonucleotides having 12 or more nucleotides are preferred. Sequences longer than 18 to 21 nucleotides may be somewhat less effective in inhibiting GBS toxin receptor translation because of decreased uptake by the target cell. Thus, oligomers of 12-21 nucleotides are most preferred in the practice of the present invention, particularly oligomers of 12-18 nucleotides. Oligonucleotides complementary to and hybridizable

with any portion of the GBS toxin receptor mRNA transcript are, in principle, effective for inhibiting translation of the transcript, and capable of inducing the effects herein described. Translation is most effectively inhibited by blocking the mRNA at a site at or near the initiation codon. Thus, oligonucleotides complementary to the 5' region of the GBS toxin receptor mRNA transcript are preferred. Secondary or tertiary structure which might interfere with hybridization is minimal in this region. Moreover, sequences that are too distant in the 3' direction from the initiation site can be less effective in hybridizing the mRNA transcripts because of a "read-through" phenomenon whereby the ribosome is postulated to unravel the antisense/sense duplex to permit translation of the message. (see, e.g. Shakin, J. Biochemistry 261, 16018 (1986)). The antisense oligonucleotide is preferably directed to a site at or near the ATG initiation codon for protein synthesis. Oligonucleotides complementary to a portion of the GBS toxin receptor mRNA including the initiation codon are preferred. While antisense oligomers complementary to the 5' region of the GBS toxin receptor transcript are preferred, particularly the region including the initiation codon, it should be appreciated that useful antisense oligomers are not limited to those complementary to the sequences found in the translated portion of the mRNA transcript, but also includes oligomers complementary to nucleotide sequences contained in, or extending into, the 5' and 3' untranslated regions. Antisense nucleotides or antisense expression constructs can find use to treat or prevent diseases associated with pathologic or hypoxia-driven angiogenesis and neovascularization, as inappropriate expression of GBS toxin receptor results in hyperproliferation of endothelial cells.

In one embodiment, the polynucleotides of the invention can exist in linear form. In another embodiment, the polynucleotides can exist in circular form as part of a plasmid.

In yet another embodiment, a probe or PCR primer comprises a group of polynucleotide species containing different degenerate codons at various positions, which polynucleotides encode, or are complementary to sequences encoding, a GBS toxin receptor in whole or in part. Such polynucleotides can be useful for isolating nucleic acid sequences encoding polypeptides having at least about 85% identity to the amino acid sequence of sheep or human GBS toxin receptor, such as, for example, GBS toxin receptors of other organisms. Typically, such polynucleotides are synthesized chemically as described above by programming a synthesizer to incorporate a particular combination of nucleic acid residues at a certain position.

Typical designations are shown in Table 2.

Table 2

Base Codes

<u>Symbol</u>	<u>Meaning</u>
A	A; adenine
C	C; cytosine
G	G; guanine
T	T; thymine
U	U; uracil
M	A or C
R	A or G
W	A or T/U
S	C or G
Y	C or T/U
K	G or T/U
V	A or C or G; not T/U
H	A or C or T/U; not G
D	A or G or T/U; not C
B	C or G or T/U; not A
N	A or C or G or T/U

POLYPEPTIDES

Another aspect of the invention provides polypeptides comprising (1) the full length GBS toxin receptor protein or a naturally occurring allelic variant thereof, (2) fragments of at least 3 amino acids of the amino acid sequence of SEQ ID NO: 2, 4, 8, 10 or 12, and (3) a GBS toxin receptor protein, polypeptide, or polypeptide fragment having an amino acid identity in the range of about 80% to 100% to the amino acid sequence of a corresponding region of SEQ ID NO: 2, 4 or 8. Preferred fragments of the amino acid sequence of SEQ ID NO: 2, 4, 8, 10 or 12, are at least 5, 6, 7, 8 or 9 amino acids in length and are immunologically reactive, i.e., immunogenic. More preferred are fragments at least 25 amino acids in length and fragments comprising the amino acid sequence of residues 181 to 419 of SEQ ID NO: 2 or residues 1 to 240 of SEQ ID NO: 4. Most preferred are fragments that can bind GBS toxin. Preferably, the GBS toxin receptor protein, polypeptide, or polypeptide fragment has an amino acid identity to the amino acid sequence of a corresponding region of SEQ ID NO: 2, 4 or 8 of at least about 86%, more preferably at least about 95% identity, even more preferably at least about 99% identity up to having one amino acid difference, and most preferably 100% identity. Preferred polypeptides have at least about 89% identity, more preferably at least about 95% identity, even more preferably at least about 99% identity up to having one amino acid difference, and most preferably 100% identity to the amino acid sequence of residues 181 to 419 of SEQ ID NO: 2, residues 1 to 495 of SEQ ID NO: 4. Preferably, a full length GBS toxin receptor protein comprises the amino acid sequence of residues 1 to 495 of SEQ ID NO: 2, residues 1 to 495 of SEQ ID NO: 4, or residues 1 to 536 of SEQ ID NO: 8, or an allelic variant thereof. The polypeptides of the invention can include amino acids in addition to the GBS toxin receptor protein, polypeptide, or polypeptide fragment. Such polypeptides typically comprise a heterologous polypeptide joined to a second polypeptide derived, as described above, from a GBS toxin receptor. Preferably the additional amino acids are covalently linked to the amino-terminal or carboxy-terminal terminus of the GBS toxin receptor protein, polypeptide, or polypeptide fragment.

Fragments or analogs of GBS toxin receptor can be prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. For example, such functional domains include domains conferring the property of induction of an inflammatory response upon binding of GBS toxin to the GBS toxin receptor. GBS toxin mediates

the binding and opsonization by C3 of endothelial cells that express the GBS toxin receptor. Such domains can comprise the binding site for GBS toxin, in whole or in part, or domains otherwise essential for GBS toxin receptor structure and/or function. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known (Bowie et al. (1991) *Science* 253: 164). Computerized prediction methods, such as, for example, a hydropathy profile as provided by the "Soap" program in PC/GENE can be employed to identify putative structural and functional domains. Using the method of Klein, Kanehisa and DeLise, Biochim Biophys Acta (1985) 815:468-476, the inventors have classified a sheep GBS toxin receptor, SP55, as an integral protein with seven transmembrane segments predicted. Such a protein is also known colloquially in the art as a "7-spanner". The predicted segments are set forth below in Table 3.

15

Table 3

Predicted Transmembrane Domains of SP55

No.	Inner Boundaries		Outer Boundaries		Segment Sequence	P:I odds*
	From	To	From	To		
1	232	248	226	252	FFGIVGIIWFILWICLV (232-248 of SEQ ID No. 4)	2.589323E-05
2	369	385	365	389	LIGMIGPAIFLVAAGFI (369-385 of SEQ ID No. 4)	1.007311E-03
3	458	474	456	479	TVFCIAAAINVFGAIF (458-474 of SEQ ID No. 4)	2.482542E-03
4	137	153	135	157	LLGFGIFATAIFTLFT (137-153 of SEQ ID No. 4)	7.564906E-03
5	42	58	42	58	LAFLSFFGFFVLYSLRV (42-58 of SEQ ID No. 4)	8.236557E-02
6	328	344	328	345	GFLSAVPYLGWLCMI L (328-344 of SEQ ID No. 4)	.1925022
7	390	406	390	407	SLAVAFLTISTTLGGFC (390-406 of SEQ ID No. 4)	.8064944

* Relates hydrophobicity of integral sequence to the hydrophobicity of the peripheral sequence. An integral sequence with a higher hydrophobicity number is more likely to be part of a transmembrane domain.

20

A computerized alignment of the amino acid sequences of GBS toxin receptor in various organisms provides further guidance in preparing preferred fragments. See, for example, Table 4 which compares the amino acid sequence of residues 42 to 536

21.

of a human GBS toxin receptor (HP59) (residues 42 to 536 of SEQ ID NO: 8) and a sheep GBS toxin receptor (SP55).

Table 4
Alignment of Human and Sheep GBS Toxin Receptor Amino Acid

5	Sequences	
SP55	MKSPVSDLAPSDGEEGSDRTPLLQRAPEAPVCCSARYNLAFLSFFGF	50
HP55	MRSPVRDLARNDGEESTDRTPLLPAPRAEAPVCCSARYNLAILAFFGF	50
SP55	FVLYSLRVNLSVALVDMVDSNTTAKDNRTSYECAHSAPIKVLHNQTGKK	100
HP55	FIVYALRVNLSVALVDMVDSNTTLEDNRTSKACPEHSAPIKVHVNQTGKK	100
SP55	YRWDAETQGWILGSFFYGYIITQIPGGYVASRSGGKLLLGFGIFATAIFT	150
HP55	YQWDAETQGWILGSFFYGYIITQIPGGYVASKIGGKMLLGFGILGTAVLT	150
SP55	LFTPLAADFGVGALVALRALEGLGEGVTYPAMHAMWSSWAPPLERSKLLS	200
HP55	LFTPIAADLGVGPLIVLRLEGLGEGVTFPAMHAMWSSWAPPLERSKLLS	200
SP55	ISYAGAQLGTIVVSLPLSGVICYYMNWTYVFYFFGIVGIIWFILWICLVSD	250
HP55	ISYAGAQLGTIVISLPLSGIICYYMNWTYVFYFFGTIGIFWFLWIVLWSD	250
SP55	TPETHKTITPYEKEYILSSLKNQLSSQKSVPWIPMLKSLPLWAIIVVAHFS	300
HP55	TPQKHKRISHYEKEYILSSLRNQLSSQKSVPWVPILKSLPLWAIIVVAHFS	300
SP55	YNWTFYTLTLLPTYMKEVLRFNQENGFLSAVPYLGWLCMILSGQAAD	350
HP55	YNWTFYTLTLLPTYMKEILRFNVQENGFLSSLPYLGSWLCMILSGQAAD	350
SP55	NLRARWNFSTLWVRRVFSLIGMIGPAIFLVAAGFIGDYSLAVAFLTIST	400
HP55	NLRAKWNFSTLCVRRIFSLIGMIGPAVFLVAAGFIGDYSLAVAFLTIST	400
SP55	TLGGFCSSGFSINHLDIAPSYAGILLGITNTFATIPGMIGPIIARSLTPE	450
HP55	TLGGFCSSGFSINHLDIAPSYAGILLGITNTFATIPGMVGPVIAKSLTPD	450
SP55	NTIGEWQTVFCIAAAINVFGAIFFTLFAKGEVQNWAI SDHQHGRN	495
HP55	NTVGEWQTVFYIAAAINVFGAIFFTLFAKGEVQNWALNDHHGHRH	495
	HP55 - SEQ ID NO: 2	
	SP55 - SEQ ID NO: 4	

Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in a GBS toxin receptor sequence.

Although one class of preferred embodiments are fragments having amino- and/or carboxy-termini corresponding to amino acid positions near functional domains borders, alternative fragments may be prepared. The choice of the amino- and carboxy-termini of such fragments rests with the discretion of the practitioner and will be made based on experimental considerations, such as ease of construction, stability to proteolysis, thermal stability, immunological reactivity, amino- or carboxyl-terminal residue modification, or other considerations. Polypeptide fragments usually contain at least nine amino acids and can contain any number of amino acids provided that the peptide fragment is at least about 80% identical to the corresponding fragment of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO:8. The human GBS toxin receptor has 41 additional amino acids on the N-terminus compared to the sheep GBS toxin receptor (compare SEQ ID NO:4 and SEQ ID NO:8). Analogs can comprise additions or deletions of some or all of those 41 N-terminal amino acids. N-terminal and C-terminal additions useful, e.g., for purification and/or antibody recognition are also contemplated. Examples include histidine tags, a FLAG (phenylalanine, leucine, alanine, guanine) epitope, fusion partners such as glutathione S transferase, chloramphenicol acetyltransferase (CAT), luciferase, β -galactosidase, and the like. Deletions of unconserved amino acids are also contemplated, provided that the structural integrity and/or binding properties of the GBS toxin receptor are not substantially compromised.

Analogs can also comprise amino acid substitutions, preferably conservative substitutions. Also preferred are conservative and/or non-conservative substitutions in regions having less shared identity among various species. For example, a variant of a GBS toxin receptor can comprise conservative and/or non-conservative substitutions of amino acids corresponding to residues 2, 6, 10, 11, 16, 17, 24, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283, 285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495 of SEQ ID NO:4. Preferably the substitution is an amino acid present in the corresponding position of SEQ ID NO:4 or SEQ ID NO:8. For example, referring to the alignment plot in Table 4, the amino acid corresponding to position 152 of SEQ ID NO:4 can be arginine (R), glutamine (Q), or a conservative or non-conservative substitution of R or Q, and preferably is R or Q. Such regions can

be identified by amino acid sequence alignment plots, such as that shown in Table 4. Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for GBS toxin, and (4) confer or modify other physicochemical or functional properties of such
5 analogs. Analogs can include various mutations of a sequence other than the naturally-occurring peptide sequence, such as, for example, single or multiple amino acid substitutions.

A conservative amino acid substitution should generally not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino
10 acid should not tend to break a helix that occurs in the parent sequence, disrupt disulfide bonds or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles*, (1984) Creighton (ed.), W.H. Freeman and Company, New York; *Introduction to Protein*
15 *Structure*, (1991), C. Branden and J. Tooze, Garland Publishing, New York, NY; and Thornton et al. (1991) *Nature* 354: 105 (which are incorporated herein by reference). A conservative substitution is a "replacement of an amino acid in a polypeptide by one with similar characteristics." (McGraw-Hill Dictionary of Scientific and
Technical Terms, Fifth Edition, 1994, Sybil P. Parker, Editor in Chief). The structure
20 and characteristics of naturally occurring amino acids has long been known in the art (Biochemistry, Second Edition, Albert L. Lehninger, 1975, pages 71-76) For example, amino acids which are similar by virtue of their hydrophobic R groups are alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, and
methionine. Alanine, valine, leucine, and isoleucine are similar by virtue of their
25 aliphatic R groups. Phenylalanine and tryptophan are similar by virtue of their aromatic R groups. Glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine are similar by virtue of their uncharged polar R groups. Glycine and alanine are similar by virtue of their small size. Serine and threonine are similar by
virtue of a hydroxyl in their R group. Asparagine and glutamine differ by only one
30 methyl group. Similarly, aspartic acid and glutamic acid differ by only one methyl group, and they are similar by virtue of their acidic R groups. Lysine, arginine, and histidine are similar by virtue of their basic R groups. In addition, lysine and arginine are similar by virtue of the amino groups on the end of the aliphatic chain in their R groups. Tyrosine and phenylalanine are similar by virtue of their aromatic groups.

Amino substitutions commonly made in the art include a substitution of valine for leucine or isoleucine, alanine for glycine, serine for threonine, asparagine for glutamine, aspartic acid for glutamic acid, and lysine for arginine, tyrosine for phenylalanine, and vice versa.

5 Typically, one skilled in the art would generally refrain from changing amino acids that are conserved among the various GBS toxin receptors, but a conservative substitution might reasonably be made. For example, Table 4 guides one skilled in the art to avoid substitutions, particularly nonconservative substitutions, for amino acids corresponding to residues 1, 3-5, 7-9, 12-15, 18-23, 26-30, 32-43, 45, 47-51, 54,
10 56-73, 76-80, 83, 85-92, 94-101, 103-131, 134-136, 138-143, 146-147, 150-154, 156-158, 160-162, 164, 167-178, 180-211, 213-218, 220-234, 237-238, 240-241, 243-245, 247-252, 255-256, 258, 261-270, 272-282, 284, 286-318, 320-323, 325-331, 334-337, 339-354, 356-361, 363-365, 367-376, 378-438, 440-441, 443-444, 446-449, 451-452, 454-460, 462-486, 489-490 and 492-494 of SEQ ID NO:4, which are conserved
15 among the GBS toxin receptors shown in Table 4.

Tables 5 and 6 describe sequences within HP59 and SP55, respectively, that match predicted amidation, N-glycosylation, cAMP-phosphorylation, CK2-phosphorylation, myristylation (addition of unsaturated fatty acid molecules), and PKC-phosphorylation sites (Omega 1.1 sequence analysis program). The information
20 contained in these tables provides guidance to one skilled in the art for designing GBS toxin receptor variants and fragments. When designing polypeptide variants, for example, one may decide to avoid substitutions in some or all of these regions. When designing polypeptide fragments other than immunogenic polypeptide fragments, for example, one may opt to include some or all of these regions.

Table 5 Putative Recognition Sites in HP59			Table 6 Putative Recognition Sites in SP55		
Site	Seq. ID NO: 8 Residues:	Sequence	Site	Seq. ID NO: 4 Residues:	Sequence
AMIDATION	23-26	SGRR	AMIDATION	97-100	TGKK
AMIDATION	138-141	TGKK	ASN_GLYCOSYLATION	59-62	NLSV
ASN_GLYCOSYLATION	100-103	NLSV	ASN_GLYCOSYLATION	71-74	NTTA
ASN_GLYCOSYLATION	112-115	NTTL	ASN_GLYCOSYLATION	77-80	NRTS
ASN_GLYCOSYLATION	118-121	NRTS	ASN_GLYCOSYLATION	95-98	NQTG
ASN_GLYCOSYLATION	136-139	NQTG	ASN_GLYCOSYLATION	225-228	NWTY
ASN_GLYCOSYLATION	266-269	NWTY	ASN_GLYCOSYLATION	302-305	NWTF
ASN_GLYCOSYLATION	343-346	NWTF	ASN_GLYCOSYLATION	357-360	NFTS
ASN_GLYCOSYLATION	398-401	NFST	CK2_PHOSPHO_SITE	11-14	SDGE
CAMP_PHOSPHO_SITE	297-300	KRIS	CK2_PHOSPHO_SITE	73-76	TAKD
CK2_PHOSPHO_SITE	113-116	TITLE	CK2_PHOSPHO_SITE	79-82	TSYE

Table 5 Putative Recognition Sites in HP59			Table 6 Putative Recognition Sites in SP55		
Site	Seq. ID NO: 8 Residues:	Sequence	Site	Seq. ID NO: 4 Residues:	Sequence
CK2_PHOSPHO_SITE	114-117	TLED	CK2_PHOSPHO_SITE	259-262	TPYE
CK2_PHOSPHO_SITE	300-303	SHYE	CK2_PHOSPHO_SITE	452-455	TIGE
CK2_PHOSPHO_SITE	493-496	TVGE	MYRISTYL	126-131	GGYVAS
MYRISTYL	66-71	GAPRAE	MYRISTYL	142-147	GIFATA
MYRISTYL	167-172	GGYVAS	MYRISTYL	162-167	GALVAL
MYRISTYL	183-188	GILGTA	MYRISTYL	172-177	GLGEGV
MYRISTYL	213-218	GLGEGV	MYRISTYL	205-210	GAQLGT
MYRISTYL	246-251	GAQLGT	MYRISTYL	209-214	GTVVSL
MYRISTYL	250-255	GTVISL	MYRISTYL	337-342	GCWLCM
MYRISTYL	378-383	GSWLCM	MYRISTYL	386-391	GCDYSL
MYRISTYL	427-432	GCDYSL	MYRISTYL	403-408	GGFCSS
MYRISTYL	444-449	GGFCSS	MYRISTYL	423-428	GILLGI
MYRISTYL	464-469	GILLGI	MYRISTYL	427-432	GITNTF
MYRISTYL	468-473	GITNTF	PKC_PHOSPHO_SITE	17-19	SDR
PKC_PHOSPHO_SITE	23-25	SGR	PKC_PHOSPHO_SITE	37-39	SAR
PKC_PHOSPHO_SITE	58-60	TDR	PKC_PHOSPHO_SITE	55-57	SLR
PKC_PHOSPHO_SITE	78-80	SAR	PKC_PHOSPHO_SITE	73-75	TAK
PKC_PHOSPHO_SITE	120-122	TSK	PKC_PHOSPHO_SITE	97-99	TGK
PKC_PHOSPHO_SITE	138-140	TGK	PKC_PHOSPHO_SITE	254-256	THK
PKC_PHOSPHO_SITE	310-312	SLR	PKC_PHOSPHO_SITE	269-271	SLK
PKC_PHOSPHO_SITE	317-320	SQK	PKC_PHOSPHO_SITE	276-278	SQK

In light of the foregoing, preferred polypeptides comprise an amino acid sequence of the formula:

AA1-AA_n-AA_m

5 wherein:

AA1 is absent or is M;

AA_n is a contiguous chain of 0 to 100 amino acids, preferably of 0 or 41 amino acids, even more preferably of residues 2-42 of SEQ ID NO:8; and

10 AA_m is a contiguous chain of 494 amino acids comprising AA43 through AA536, wherein:

(1) each of AA43, AA47, AA51, AA52, AA57, AA58, AA65, AA66, AA72, AA85, AA87, AA93, AA94, AA96, AA115, AA116, AA122, AA123, AA125, AA134, AA143, AA173, AA174, AA178, AA185, AA186, AA189, AA190, AA196, AA200, AA204, AA206, AA207, AA220, AA253, AA260, AA276, AA277, AA280, AA283, AA287, AA294, AA295, AA298, AA300, AA301, AA312, AA324, AA326, AA360, AA365, AA373, AA374, AA379, AA396, AA403, AA407, AA418, AA480, AA483, AA486, AA491, AA494, AA502, AA528, AA529, AA532 and AA536 is an essential amino acid or a modified amino acid and preferably is an amino acid residue corresponding to:

25 (a) residue 43, 47, 51, 52, 57, 58, 65, 66, 72, 85, 87, 93, 94, 96, 115, 116, 122, 123, 125, 134, 143, 173, 174, 178, 185, 186, 189, 190, 196, 200, 204, 206, 207, 220, 253, 260, 276, 277, 280, 283, 287, 294, 295, 298, 300, 301, 312, 324, 326, 360, 365, 373, 374, 379, 396, 403, 407, 418, 480, 483, 486,

491, 494, 502, 528, 529, 532 and 536, respectively, of SEQ ID NO:8;

(b) residue 2, 6, 10, 11, 16, 17, 24, 25, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283, 285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495, respectively of SEQ ID NO:4; or

(c) a conservative substitution thereof;

(2) each of AA44-AA46, AA48-AA50, AA53-AA56, AA59-AA64, AA67-AA71, AA73-AA84, AA86, AA88-AA92, AA95, AA97-AA114, AA117-AA121, AA124, AA126-AA133, AA135-AA142, AA144-AA172, AA175-AA177, AA179-AA184, AA187-AA188, AA191-AA195, AA197-AA199, AA201-AA203, AA205, AA208-AA219, AA221-AA252, AA254-AA259, AA261-AA275, AA278-AA279, AA281-AA282, AA284-AA286, AA288-AA293, AA296-AA297, AA299, AA302-AA311, AA313-AA323, AA325, AA327-AA359, AA361-AA364, AA366-AA372, AA375-AA378, AA380-AA395, AA397-AA402, AA404-AA406, AA408-AA417, AA419-AA478, AA481-AA482, AA484-AA485, AA487-AA490, AA492-AA493, AA495-AA501, AA503-AA527, AA530-AA531 and AA533-AA535 is

(a) residue 44-46, 48-50, 53-56, 59-64, 67-71, 73-84, 86, 88-92, 95, 97-114, 117-121, 124, 126-133, 135-142, 144-172, 175-177, 179-184, 187-188, 191-195, 197-199, 201-203, 205, 208-219, 221-252, 254-259, 261-275, 278-279, 281-282, 284-286, 288-293, 296-297, 299, 302-311, 313-323, 325, 327-359, 361-364, 366-372, 375-378, 380-395, 397-402, 404-406, 408-417, 419-478, 481-482, 484-485, 487-490, 492-493, 495-501, 503-527, 530-531 and 533-535, respectively, of SEQ ID NO:8; or

(b) a conservative substitutions thereof; and

(3) AA315 through AA367 are optionally absent.

Preferred polypeptides comprise the amino acid sequence of SEQ ID NO:4, SEQ ID NO:8 or an amino acid sequence which varies from that sequence only at the specific residues which are not conserved between the sheep GBS toxin receptor (SEQ ID NO:4) and the human GBS toxin receptor (SEQ ID NO:8). Of those variations, the most preferred variations are those resulting in a polypeptide encoded by SEQ ID NO:11. Even more preferred variations are those amino acids in the corresponding positions of the amino acid sequence of SEQ ID NO:4. Particularly preferred are polypeptides comprising an amino acid sequence that differs from SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:8 at no more than about 20% of the amino acid residues, with increasing preference for no more than about 10%, 5%, 1%, with one to zero amino acid differences being most preferred.

Besides targeting specific amino acids for change, analogs of GBS toxin receptor can also be prepared by techniques involving activity selection, such as, for example, phage display, directed evolution, DNA shuffling, and homologous in procaryotes or eucaryotes of genes from different species, as described in part in U.S. Patent Nos. 5,605,793; 5,830,721; 5,811,238; 5,837,458; 5,093,257; 5,223,409; 5,403,484; 5,571,698; and 5,837,500, which are incorporated herein by reference.

Any variant or fragment of the human and sheep GBS toxin receptors described herein can be tested for the requisite activity by determining whether the variant or fragment can bind GBS toxin.

10 These polypeptides provide reagents useful in drug discovery and purification and can be used in various *in vitro* assays, preferably when expressed on the surface of a cell, e.g., a stable transfected cell. For example, assays such as binding assays can be used to screen test compounds, including polysaccharides and other compounds, for their ability to bind the GBS toxin receptor. Assays can identify
15 potential drug candidates that block GBS toxin binding to the GBS toxin receptor. Such drugs are useful for preventing and/or treating early onset disease in neonatal humans. Some polypeptides can be used to competitively inhibit binding GBS toxin to a GBS toxin receptor.

The polypeptides of the invention can be used to affinity purify GBS toxin, a GBS toxin chimeric compound, and other polysaccharides or compounds which can bind the GBS toxin receptor.

- The polypeptides can also be used to develop a method of targeting a cytotoxic agent for delivery to a cell that expresses a GBS toxin receptor. For example, a cytotoxic agent can be coupled to a molecule that binds a GBS toxin receptor for selective delivery to the neovasculature of a growing tumor. Such a delivery system would permit a highly concentrated, localized attack on a growing tumor, while minimizing the adverse systemic side effects encountered with most chemotherapeutics. In one instance, the cytotoxic agent can be GBS toxin, which, upon binding to GBS toxin receptor, induces an inflammatory response as described in Hellerqvist et al., Angiogenesis: Molecular Biology, Clinical Aspects, Edited by M.E. Maragoudakis et al., Plenum Press, New York 1994, pp. 265-269. In a similar manner, selective delivery of a therapeutic agent to a cell that expresses a GBS toxin receptor could be used advantageously to treat tumors, rheumatoid arthritis or neural injury, or to facilitate wound healing.

The polypeptides of the invention can also be used to screen for and/or design a GBS toxin mimetic with improved therapeutic properties, such as, for example, improved ability to inhibit hypoxia-induced neovascularization or angiogenesis. Such mimetics are useful in the treatment and prevention of conditions resulting from hypoxia-induced neovascularization or angiogenesis, such as, for example, tumor growth, scarring during wound healing, gliosis during repair of neural injury, reperfusion injury, restenosis, rheumatoid arthritis, psoriasis, other chronic inflammatory diseases characterized by angiogenesis, etc. Therapeutic properties can be improved by enhancing biological stability, affinity for the GBS toxin receptor, complement binding activity, reducing antigenicity, etc.

The polypeptides of the invention can also be used to generate antibodies for various therapeutic and research purposes. The polypeptides of the invention can be used to immunize rabbits, mice, goats, chickens, or other animals known in the art to be amenable to such immunization. Monoclonal antibodies are generally preferred but polyclonal antibodies can also be used, provided that detection of binding of the GBS toxin receptor antibody to the GBS toxin receptor is possible. The production of non-human monoclonal antibodies, e.g., murine, is well known (see, e.g., Harlow et al., *Antibodies A Laboratory Manual*, Cold Spring Harbor Press, pp. 139-240, 1989, 29.

incorporated herein by reference). As it may be difficult to generate human monoclonal antibodies to a human receptor or binding domain polypeptide, it may be desirable to transfer antigen binding regions of non-human monoclonal antibodies, e.g. the F(ab')₂ or hypervariable regions or murine monoclonal antibodies, to human constant regions (Fc) or framework regions by recombinant DNA techniques to produce substantially human molecules. Such methods are generally known and are described in, e.g., U.S. Pat. Nos. 4,816,397 and 4,946,778, and EP publications 173,494 and 239,400. Alternatively, one may isolate DNA sequences which code for a human monoclonal antibody or portions thereof that specifically bind to the receptor protein by screening a DNA library from human B cells according to the general protocol outlined in WO 90/14430, and then cloning and amplifying the sequences which encode the antibody (or binding fragment) of the desired specificity.

Usually, polypeptides used for producing antibodies are the full-length receptor or receptor fragments designed from putative extracellular domains identified by a variety of methods known in the art, including computer programs which predict secondary and tertiary structure of a polypeptide based upon its primary amino acid sequence. Another method for designing antigenic peptides utilizes computer programs that predict the high points of hydrophilicity within a particular primary amino acid sequence. For example, using the method of Happ and Woods, *Proc. Natl. Acad. Sci. USA* (1981) 78:3824-3829, via the "Antigen" program in PC/GENE, the inventors identified 3 regions of high hydrophilicity, shown below in Table 7, and used the results to design antigenic peptides to be used in the preparation of antibodies against GBS toxin receptor (see Example 4).

Table 7

High Points of Hydrophilicity in SP55

No.	Ah	Sequence
1	2.05	Glu-Glu-Gly-Ser-Asp-Arg (14-19 of SEQ ID No. 2)
2	1.52	Lys-Asp-Asn-Arg-Thr-Ser (75-80 of SEQ ID No. 2)
3	1.33	Arg-Ala-Pro-Arg-Ala-Glu (25-30 of SEQ ID No. 2)

Ah = Average hydrophilicity.

Antibodies that recognize various portions of the intact GBS toxin receptor can be used to further investigate structure and function of the receptor. The polypeptides of the invention can give rise to antibodies that recognize a variety of forms of GBS toxin receptor, including, but not limited to, intact GBS toxin receptor

expressed on a cell surface, denatured GBS toxin receptor or non-denatured GBS toxin receptor, and GBS toxin receptor purified away from cellular components or GBS toxin receptor contained in a cell lysate. GBS toxin receptor antibodies can be used to study species differences as well as GBS toxin receptor expression levels in

5 various cell types.

Antibodies that recognize a portion or all of an extracellular domain are particularly useful as a diagnostic for the monitoring of tumor growth and metastasis, for the detection or identification of a chronic inflammatory condition, such as, for example, rheumatoid arthritis or psoriasis, and for the detection of other medical conditions arising due to hypoxia-driven angiogenesis, such as, for example, restenosis. Typically, such antibodies can be employed in a variety of standard research and diagnostic techniques, including, but not limited to, western blot, immunoprecipitation, ELISA, radioimmunoassay (RIA), BIACOR®, enzyme-linked-immunoassay (EIA), immunofluorescence, fluorescence activated cell sorting (FACS), and *in vivo* diagnostic imaging systems such as magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR), computerized axial tomography (CAT) scan, and position emission tomography (PET), etc.

In addition, antibodies that block the binding of GBS toxin to a GBS toxin receptor can be used for the treatment or prevention of early onset disease in a neonatal human. Such antibodies can directly or indirectly block the GBS toxin binding site on the GBS toxin receptor.

In one embodiment, the GBS toxin receptor protein is naturally occurring and can be isolated from a cell extract by protein purification techniques known in the art, such as, for example, ion exchange column chromatography, high performance liquid chromatography (HPLC), reversed phase HPLC, or affinity chromatography using antibodies that recognize the GBS toxin receptor.

Alternatively, the isolated proteins and polypeptides are expressed using polynucleotides encoding the polypeptide(s) of the invention in operative association with an appropriate control sequence including a promoter in an expression vector suitable for expression, preferably in a mammalian cell, and also in bacterial, insect, or yeast cells.

Usually, the GBS toxin receptor polynucleotide or a fragment thereof can be expressed in a mammalian system. Such expression will usually depend on a mammalian promoter, which is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. Usually, a promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region typically includes an RNA polymerase binding site and a transcription initiation site.

Vectors suitable for replication in mammalian cells are known in the art, and can include viral replicons, or sequences that ensure integration of the sequence encoding PAK65 into the host genome. Suitable vectors can include, for example, those derived from simian virus SV40, retroviruses, bovine papilloma virus, vaccinia virus, and adenovirus.

A suitable vector, for example, is one derived from vaccinia viruses. In this case, the heterologous DNA is inserted into the vaccinia genome. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art, and utilize, for example, homologous recombination. The insertion of the heterologous DNA is generally into a gene which is non-essential in nature, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid shuttle vectors that greatly facilitate the construction of recombinant viruses have been described (see, for example, Mackett et al. (1984); Chakrabarti et al. (1985); Moss (1987)). Expression of the heterologous polypeptide then occurs in cells or individuals which are immunized with the live recombinant vaccinia virus.

Such suitable mammalian expression vectors usually contain one or more eukaryotic transcription units that are capable of facilitating expression in mammalian cells. The transcription unit is comprised of at least a promoter element to mediate transcription of foreign DNA sequences. Suitable promoters for mammalian cells are known in the art and include viral promoters such as those from simian virus 40 (SV40) (Subramani et al., Mol Cell. Biol. 1:854-864, 1981), cytomegalovirus (CMV) (Boshart et al., Cell 41:521-530, 1985), Rous sarcoma virus (RSV), adenovirus (ADV) (Kaufman and Sharp, Mol. Cell. Biol. 2:1304-1319, 1982), and bovine papilloma virus (BPV), as well as cellular promoters, such as a mouse metallothionein-1 promoter (U.S. Patent No. 4,579,821), a mouse VK promoter (Bergman et al., Proc. Natl. Acad. Sci. USA 81:7041-7045, 1993; Grant et al., Nuc. Acids Res. 15:5496, 1987), and a mouse VH promoter (Loh et al., Cell 33:85-93, 1983).

The optional presence of an enhancer element (enhancer), combined with the promoter elements described herein, will typically increase expression levels. An enhancer is any regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to endogenous or heterologous promoters, with synthesis beginning at the normal mRNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or

flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) Science 236:1237; Alberts et al. (1989) Molecular Biology of the Cell, 2nd ed.). Enhancer elements derived from viruses can be particularly useful, because they typically have a broader host range. Examples useful in mammalian
5 cells include the SV40 early gene enhancer (Dijkema et al (1985) EMBO J. 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) Proc. Natl. Acad. Sci. 79:6777), from human cytomegalovirus (Boshart et al. (1985) Cell 41:521) as well as the mouse μ enhancer (Gillies, Cell 33:717-728, 1983). Additionally, some enhancers are regulatable and
10 become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) Trends Genet. 2:215; Maniatis et al. (1987) Science 236:1237).

In addition, the transcription unit can also be comprised of a termination sequence and a polyadenylation signal which are operably linked to the GBS toxin
15 receptor coding sequence. Polyadenylation signals include, but are not limited to, the early or late polyadenylation signals from SV40 (Kaufman and Sharp), the polyadenylation signal from the Adenovirus 5 E1B region and the human growth hormone gene terminator (DeNoto et al., Nuc. Acids Res. 9:3719-3730, 1981).

Sequences that cause amplification of the gene may also be desirable, as are
20 sequences which encode selectable markers. Selectable markers for mammalian cells are known in the art, and include, for example, thymidine kinase, dihydrofolate reductase (together with methotrexate as a DHFR amplifier), aminoglycoside phosphotransferase, hygromycin B phosphotransferase, asparagine synthetase, adenosine deaminase, and antibiotic resistant genes such as neomycin.

25 A GBS toxin receptor, or fragment thereof, can be expressed on the surface of a cell, or can be expressed in soluble or secreted form. Expression on the surface of the cell can be achieved, for example, by including a secretory leader operably linked to a nucleic acid sequence encoding the desired receptor fragment and at least one transmembrane domain. The secretory leader can be that encoded by the GBS toxin
30 receptor gene, or can be a heterologous leader sequence commonly used in the art, such as, for example, the leader sequence of Schizosaccharomyces pombe pho1+ acid phosphatase (Braspenning et al., Biochem Biophys Res. Commun (1998) 245:166-71), the leader sequence of human interleukin-2 (IL-2) gene (Sasada et al., Cell Struct

Funct (1988) 13:129-141). Expression in soluble or secreted form can be achieved, for example, by excluding from the gene construct nucleic acid sequences encoding a transmembrane domain. In some instances, solubility and/or secretion are achieved by the use of a fusion partner, such as, for example, chloramphenicol acetyltransferase (CAT), β -galactosidase, and other genes readily expressed in the selected host cell.

The vector that encodes GBS toxin receptor can be used for transformation of a suitable mammalian host cell. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (these patents are incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), N1E-115 (Liles et al., J. Biol. Chem. 261:5307-5313, 1986), PC 12 human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines, such as insect derived cell lines IF9 and IF21. Cell lines of particular preference are those expressing recombinant GBS toxin receptor constructs constitutively, lines which subsequently develop characteristics of a transformed cell, and lines which more preferably express GBS toxin receptor or fragments on the cell surface. Particularly preferred are ECV cells (a bladder carcinoma cell line originally referred to in the scientific literature as an endothelial cell line), human umbilical vein endothelial cells (HUVEC), bovine, sheep, and human adrenal medulla endothelial cells.

Recombinant GBS toxin receptor or fragments thereof can be produced by culturing host cells expressing the receptor or fragment in a suitable culture medium and under appropriate cell culture conditions. Culture media and conditions are

variable depending on the requirements of a particular host cell line and are well known in the art. Typically, cells are cultured at 37°C in a cell culture incubator with a fixed amount of CO₂, usually in the range of 5-10%.

In another embodiment, the polypeptide fragments can be synthesized chemically by techniques well known in the art, such as solid-phase peptide synthesis (Stewart et al., Solid Phase Peptide Synthesis, W.H. Freeman Co., San Francisco (1963)); Merrifield, J Am Chem Soc 85:2149-2154 (1963)). These and other methods of peptide synthesis are also exemplified by U.S. Patent Nos. 3,862,925, 3,842,067, 3,972,859, and 4,105,602. The synthesis can use manual synthesis techniques or automatically employ, for example, an Applied BioSystems 430A or 431A Peptide Synthesizer (Foster City, California) following the instructions provided in the instruction manual supplied by the manufacturer. It will be readily appreciated by those having ordinary skill in the art of peptide synthesis that the intermediates which are constructed during the course of synthesizing the present analog compounds are themselves novel and useful compounds and are thus within the scope of the invention.

In addition to polypeptides consisting only of naturally-occurring amino acids, peptidomimetics are also provided. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (Fauchere, J. (1986) Adv. Drug Res. 15: 29; Veber and Freidinger (1985) TINS p.392; and Evans et al. (1987) J. Med. Chem 30: 1229, which are incorporated herein by reference) and are usually developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity) but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: -CH₂NH-, -CH₂S-, -CH₂-CH₂-, -CH=CH- (cis and trans), -COCH₂-, -CH(OH)CH₂-, and -CH₂SO-, by methods known in the art and further described in the following references: Spatola, A.F. in "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Morley, J.S., Trends Pharm Sci (1980) pp. 463-468 (general

review); Hudson, D. et al., *Int J Pept Prot Res* (1979) 14:177-185 (-CH₂NH-, CH₂CH₂-); Spatola, A.F. et al., *Life Sci* (1986) 38:1243-1249 (-CH₂-S); Hann, M.M., *J Chem Soc Perkin Trans I* (1982) 307-314 (-CH-CH-, cis and trans); Almquist, R.G. et al., *J. Med Chem* (1980) 23:1392-1398 (-COCH₂-); Jennings-
5 White, C. et al., *Tetrahedron Lett* (1982) 23:2533 (-COCH₂-); Szelke, M. et al., *European Appln. EP 45665* (1982) CA: 97:39405 (1982) (-CH(OH)CH₂-); Holladay, M.W. et al., *Tetrahedron Lett* (1983) 24:4401-4404 (-C(OH)CH₂-); and Hruby, V.J., *Life Sci* (1982) 31:189-199 (-CH₂-S-); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is -CH₂NH-. Such peptide
10 mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others. Labeling of peptidomimetics usually involves covalent attachment of one or
15 more labels, directly or through a spacer (e.g., an amide group), to non-interfering position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with GBS toxin (e.g., are not contact points in the GBS toxin binding domain of the GBS toxin receptor). Derivatization (e.g.,
20 labelling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic.

Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a
25 consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch (1992) *Ann. Rev. Biochem.* 61: 387, incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

30 The invention also provides a complex comprising a GBS toxin bound to a mammalian GBS toxin receptor or a fragment of a mammalian GBS toxin receptor. Preferably, the complex comprises a GBS toxin bound to a GBS toxin receptor polypeptide described above that can bind GBS toxin. Typically, a complex is formed by contacting a GBS toxin with such a polypeptide under conditions that

permit specific binding of the GBS toxin to the polypeptide. The GBS toxin can be labeled or unlabeled. The polypeptide can be present on the surface of a cell, or immobilized in a well or on a bead, or the polypeptide can be present in solution.

5 DETECTION METHODS

Yet another aspect of the invention provides methods for detecting or monitoring a variety of medical conditions characterized by pathologic and/or hypoxia-driven angiogenesis or neovascularization. Examples include, but are not limited to, early onset disease in the neonate, and the progression of cancers involving
10 tumors.

Early onset disease can be diagnosed by detecting the presence or absence of GBS toxin in a patient. One method of detection is a competition assay that determines the effect of a suspected sample on the formation of a complex between GBS toxin and a GBS toxin receptor or fragment thereof. For example, the method
15 comprises contacting a control GBS toxin with a GBS toxin receptor polypeptide, in the presence and absence of a sample suspected of containing GBS toxin and under conditions that permit specific binding of the GBS toxin to the polypeptide, and comparing the amount of complex formation achieved in the presence of the suspected sample to the amount of complex formation achieved in the absence of the
20 suspected sample. Preferably, the control GBS toxin is substantially purified and of a known concentration. Preferably, the control GBS toxin further comprises a label. Suitable labels include, but are not limited to, radioisotopes, chromophores, fluorophores, biotin, avidin, and other labels used by one skilled in the art. Another method directly measures, rather than by competition with a control GBS toxin,
25 complex formation between GBS toxin present in a suspected sample and a GBS toxin receptor polypeptide.

Pathologic vasculature can be detected in a mammalian tissue by detecting the presence or absence of GBS toxin receptor in the region of a tumor, with the presence of GBS toxin receptor being indicative of the presence of pathologic vasculature. The
30 method can be used to monitor tumor growth or metastasis. One method of detection involves the use of molecules, e.g. antibodies, that specifically bind to a GBS toxin receptor, preferably an extracellular domain of GBS toxin receptor. Typically, the method comprises administering, to a mammalian tissue, e.g. in a mammal having a cancerous tumor, e.g., an antibody that recognizes a GBS toxin receptor, and

detecting specific binding of the antibody. Typically, the antibody is a labeled antibody. Preferably, the observations are quantitative and can be visual.

During surgery, the margin of a tumor can be visualized by any of a number of imaging techniques known in the art and described above. The imaging of the tumor is effected by detecting the binding of a labeled antibody or other molecules to the GBS toxin receptor on the pathologic vasculature of a tumor. This type of surgery is also known as virtual surgery because while performing the surgery, the surgeon views the tumor indirectly on an imaging screen.

10 DRUG DISCOVERY

A fourth aspect of the invention provides methods, using the polypeptides of the invention, of identifying drug candidates for the treatment of medical conditions characterized by hypoxia-driven angiogenesis or neovascularization. Preferred compounds are competitive inhibitors of GBS toxin binding to a GBS toxin receptor or inhibit GBS toxin receptor activity. Particularly preferred are compounds that inhibit the first phosphorylation step in the signal transduction pathway. Compounds can be produced by a variety of random drug design methods commonly known in the art, such as, for example, combinatorial chemistry (U.S. Patent No. 5,646,285; U.S. Patent No. 5,639,603), peptide libraries (U.S. Patent No. 5,591,646; U.S. Patent No. 5,367,053; U.S. Patent No. 5,747,334), phage display (U.S. Patent No. 5,403,484; U.S. Patent No. 5,223,409), SELEX® (U.S. Patent No. 5,773,598; U.S. Patent No. 5,763,595; U.S. Patent No. 5,763,566), and combinatorial carbohydrate chemistry (Hirschmann et al., J Med Chem (1996) 39:2441-2448; Hirschmann et al., J Med Chem (1998) 41:1382-1391; Sofia MJ, Mol Divers (1998) 3:75-94; U.S. Patent No. 5,780,603; U.S. Patent No. 5,756,712)

An alternative approach is rational drug design with the intent of producing a GBS toxin mimetic or a GBS toxin receptor mimetic with improved therapeutic properties using techniques such as x-ray crystallography, nuclear magnetic resonance (NMR) correlation spectra (U.S. Patent No. 5,698,401), computer assisted molecular modeling (U.S. Patent No. 5,579,250; U.S. Patent No. 5,612,895; U.S. Patent No. 5,680,331, Cooper et al., J. Comput.-Aided Mol. Design, 3:253-259 (1989); Brent et al., J. Comput.-Aided Mol. Design 2:311-310 (1988)) and other methods of rational drug design known in the art. FIG. 1 provides a broad overview of some of the main steps in some of the rational drug design methods of the present invention. For

example, one approach to rational drug design involves a computer program, such as INSIGHTII (available from Bisoym Technologies, 10065 Barnes Canyon Road, San Diego, California) to identify active sites in proteins by homology-based modeling. This method facilitates the modeling of a protein by using a similar protein whose structure is well known. Commercial software containing search algorithms for three dimensional database comparisons are available from vendors such as Day Light Information Systems, Inc., Irvine, California 92714, and Molecular Design Limited, 2132 Faralton Drive, San Leandro, California 94577.

In one embodiment, the compound can bind the GBS toxin receptor and induce an inflammatory response in a manner similar to the binding of GBS toxin to the GBS toxin receptor. Such compounds can be used, for example, as a drug to target an inflammatory response to the developing vasculature of a tumor.

In another embodiment, the compound can bind the GBS toxin receptor with or without inducing an inflammatory response, preferably without inducing an inflammatory response. In one instance, the compound can be used as a vehicle to target pathological neovasculature for treatment with a cytotoxic agent. For example, the cytotoxic agent can be chemically coupled to the compound to form a chimeric drug. Such chimeric drugs can be used in the treatment of tumors, rheumatoid arthritis, wound healing, spinal cord injury, and other conditions characterized by hypoxia-driven angiogenesis or neovascularization. In another instance, the compound can be used directly to competitively inhibit binding of GBS toxin to a GBS toxin receptor. Such compounds can be used in the treatment of early-onset disease in the neonate.

In a third embodiment, the compound can bind GBS toxin and can be used in the treatment of early-onset disease in the neonate.

The polynucleotides of the invention can be expressed in random mutagenesis systems such as phage display or the yeast two-hybrid system for the synthesis and identification of mutant peptide GBS toxin receptor polypeptides that bind GBS toxin. Alternatively, immobilized or soluble GBS toxin receptor fragments of the invention can be used to screen combinatorial peptide and combinatorial chemical libraries and non-random recombinant and synthetic peptides and other compounds (such as non-peptide molecules) for GBS toxin receptor binding. Compounds that bind GBS toxin or GBS toxin receptor can then be further characterized in a functional assay for any of the activities described above in order to identify a drug candidate for the treatment

of medical conditions involving angiogenesis or neovascularization.

A compound which inhibits binding of GBS toxin to a GBS toxin receptor can be identified by combining a test compound with a mammalian GBS toxin receptor or fragment thereof capable of binding GBS toxin, under conditions that permit specific
5 binding of GBS toxin to the GBS toxin receptor or fragment, and determining the amount of inhibition by the compound of the binding of GBS toxin to the GBS toxin receptor or fragment.

In a preferred embodiment, the GBS toxin receptor or fragment is expressed by a cell, preferably on the cell surface. The cells are contacted with labeled GBS
10 toxin in the presence or absence of the test compound. A change in the binding of GBS toxin to the GBS toxin receptor is then determined. Alternatively, the GBS toxin is unlabeled and an antibody that recognizes GBS toxin is labeled instead. The labeled antibody is used to measure inhibition by a compound of GBS toxin binding to the GBS toxin receptor or fragment. In another embodiment, the GBS toxin
15 receptor or fragment is not associated with a cell, but is instead coupled to a matrix, such as, for example, a well in a microtiter plate or a bead. Additional suitable solid supports include latex, polystyrene beads (Interfacial Dynamics Corp. Portland, Oreg.), magnetic particles (Advanced Magnetics, Cambridge, Mass.) and nylon balls (Hendry et al., *J. Immunological Meth.*, 35:285-296, 1980). The receptor or fragment
20 can be coupled to the matrix directly or indirectly through an antibody, coupled to the matrix, that binds the receptor fragment. In a third embodiment, the GBS toxin receptor or fragment is soluble and can be immunoprecipitated with an antibody that recognizes the receptor or fragment.

A preferred method for identifying a compound which binds a mammalian
25 GBS toxin receptor comprises the steps of (1) combining a test compound with a GBS toxin receptor or fragment thereof under conditions that allow specific binding to occur, and (2) detecting a complex formed between the test compound and the GBS toxin receptor or fragment. A preferred method is a competition assay which determines the ability of the test compound to compete for binding to the GBS toxin
30 receptor or fragment. In such an assay, GBS toxin is combined with the GBS toxin receptor or fragment in the presence or absence of the test compound. Decreased specific binding of GBS toxin in the presence versus the absence of the test compound is indicative of the ability of the test compound to bind a mammalian GBS toxin receptor. Another method comprises combining a control compound with the GBS

toxin receptor or fragment under the same conditions as the test compound and comparing the amount of complex formation between the test compound or the control compound and the GBS toxin receptor or fragment thereof. Preferably, the test compound and/or the control compound are labeled. The test compound can be
5 any of a number of classes of compounds, such as for example, small organic molecules (such as those used for and obtained by combinatorial chemistry), polysaccharides, polypeptides, RNA, antibodies, and single chain antibodies. In a preferred embodiment, the polypeptide is expressed by a cell, preferably on the surface of the cell, and preferably by a stable transfected cell. Such a system is
10 particularly useful for testing the effectiveness of a chimeric compound comprising a cytotoxic agent. The cytotoxic activity of the compound can be determined by exposing a cell expressing the GBS toxin receptor on the cell surface to the test chimeric compound and detecting signs of cytotoxicity. One could detect such signs by a viability stain of the cell, by detecting apoptosis (for example, by a DNA ladder
15 assay or a TUNEL™ stain, which binds to broken DNA), by measuring tritiated thymidine incorporation into the cell, and by quantitating kinase-dependent phosphorylation (e.g., using phosphoantibodies or various phosphoimaging techniques).

In another embodiment, the invention provides a method for identifying an
20 inhibitor of GBS toxin receptor. The method comprises incubating test cells in the presence and absence of a test compound. The test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity (e.g., a fragment that increases the proliferation or migration of the expressing cells relative to control cells of the same cell type that do not express the fragment). The test cells are incubated
25 under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate. Control cells that do not express the GBS toxin receptor or fragment proliferate or migrate less than cells that express the GBS toxin receptor or fragment. The proliferation or migration (also referred to herein as motility) of the test cells incubated in the presence or absence of the test compound is compared. Less
30 proliferation or migration in the presence of the test compound than in the absence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor. Preferably, as a control to determine whether the test compound specifically inhibits the GBS toxin receptor, the proliferation or migration of control cells in the presence and absence of the test compound is also compared. In the

- absence of a difference in the proliferation or migration of control cells incubated in the presence or absence of the test compound, decreased proliferation or migration in test cells exposed to the test compound relative to test cells not exposed to the test compound is indicative of specific inhibition of the GBS toxin receptor. It will be readily apparent that the control portions of the method need not be performed contemporaneously with the test portions of the method. For example, control cells can be incubated with a battery of test compounds to determine cellular effects of the test compounds prior to incubating the test cells with the test compounds. Motility or migration can be determined by detecting movement of cells on a culture dish.
- 5 Proliferation can be detected in a number of ways, including, but not limited to, measuring tritiated thymidine incorporation, cell counts, apoptosis assays, and viability assays. Preferred cells include cells transfected with GBS toxin receptor, preferably endothelial cells transfected with GBS toxin receptor, even more preferably vascular endothelial cells or microvascular endothelial cells. Primary cells
- 10 that express GBS toxin receptor are also preferred, for example, endothelial cells that have been passaged in cell culture, at confluence, no more than 8 or 9 times. A preferred class of test compounds includes kinase inhibitors, preferably cAMP-dependent kinase inhibitors, PKC inhibitors, and CK2 inhibitors, which can be used as a starting point for developing more specific GBS toxin receptor inhibitors.
- 15 Another class of compounds includes antibodies specific for GBS toxin receptor. Particularly preferred are single chain antibodies, preferably a collection of single chain antibodies that recognize various epitopes on the GBS toxin receptor. Less preferred are divalent antibodies specific for the binding site of the GBS toxin receptor ligand because they may trigger the signal transduction cascade upon
- 20 dimerization.
- 25

Another embodiment of the invention is a method of identifying an inhibitor of endothelial cell proliferation or migration, which are essential components of angiogenesis. The method basically comprises the steps described in the preceding paragraph and uses endothelial cells.

- 30 Yet another embodiment of the invention is a method of identifying a therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic or hypoxia-driven angiogenesis or neovascularization. The method basically comprises the steps described above and uses cells from tissues derived from mammals afflicted with the medical condition or cells that serve as a

model for afflicted tissue.

A preferred method for designing a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor comprises (1) simulating and selecting the most probable conformations of a GBS toxin receptor or fragment thereof, (2) designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the GBS toxin receptor or fragment, (3) chemically synthesizing the analog, and (4) evaluating the bioactivity of the analog. Preferably, steps (a) and (b) are performed with the aid of a computer program.

10 A preferred method for designing a compound which binds to a mammalian GBS toxin receptor comprises (1) simulating and selecting the most probable conformations of a GBS toxin receptor or fragment thereof, (2) deducing most probable binding domains of the receptor or fragment, (3) designing a compound that would form the energetically most probable complexes with the receptor or fragment, 15 (4) chemically synthesizing the compound, and (5) evaluating the bioactivity of the compound. Preferably, steps (a)-(c) are performed with the aid of a computer program.

Preferred polypeptides for use in the screening assays described above are polypeptides sharing at least about 85% identity, preferably at least about 95% 20 identity, and most preferably greater than about 99% identity with the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment thereof having GBS toxin receptor activity. Most preferred are polypeptides having an amino acid sequence of SED ID NO: 2, 4 OR 8 or a fragment thereof having GBS toxin receptor activity.

25 METHODS OF PURIFICATION

Another aspect of the invention is a method for purifying a compound that binds a GBS toxin receptor, for example, natural ligand, other polysaccharides, or an antibody specific for the GBS toxin receptor. The method comprises providing a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that 30 binds GBS toxin, contacting the polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide, and separating the bound compound from the remainder of the sample. The polypeptide can be soluble but preferably is immobilized on a substrate e.g., on a bead, membrane or on the surface of a cell, preferably a stable transfected cell.

METHODS OF TREATMENT

GBS toxin receptor polypeptides and antibodies that interfere with GBS toxin binding can be used in a method of treatment of the human or animal body. For example, such inhibitors of GBS toxin binding can be administered to a patient to

5 treat or prevent medical conditions involving GBS toxin binding to a GBS toxin receptor, such as, for example, early onset disease in the neonate.

GBS toxin mimetics or other compounds that bind and/or inhibit GBS toxin receptor, some of which can be identified by the drug discovery assays of the invention, can be used in a method of treatment of the human or animal body or can be used for the manufacture of a medicament for the treatment or prevention of any of a number of medical conditions involving pathologic and/or hypoxia-driven angiogenesis, such as, for example, cancerous tumors, chronic inflammatory diseases, scarring during wound healing or repair of neural injury.

In a preferred embodiment, such a compound exerts its therapeutic effect by binding GBS toxin receptor and evoking an inflammatory response, as does GBS toxin. Preferably, such compounds comprise a sulfhydryl, hydroxyl, or amino group displayed so as to be available for binding complement C3.

In another preferred embodiment, the compound is an inhibitor of GBS toxin activity. Preferred inhibitors include, but are not limited to, kinase inhibitors, single chain antibodies specific for the GBS toxin receptor, and antisense polynucleotides that specifically hybridize under high stringency conditions to a GBS toxin receptor nucleic acid sequence, such as that of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:7.

In another preferred embodiment, the compound exerts its therapeutic effect without evoking an inflammatory response. The compound can be used to deliver a cytotoxic agent to tissue in close proximity to a cell expressing a GBS toxin receptor, such as, for example, a tumor undergoing angiogenesis. Preferably, the compound is covalently attached to a cytotoxic agent and can be associated non-covalently with a cytotoxic agent, such as, for example, on the external surface of a liposome, micelle, or other lipophilic drug encapsulating structure. Preferred cytotoxic agents include antineoplastic agents commonly known in the art, such as, for example, mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide, and other alkylating agents, methotrexate and other folate antagonists, 6-mercaptopurine and other purine antagonists, 5-fluorouracil and other pyrimidine antagonists, cytarabine, ovinblastine, vincustine, and other vincas, etoposide and other podophyllotoxins, doxorubicin, bleomycin, mitomycin, and other antibiotics, carmustine, lomustine and other nitrosureas, cisplatin, interferon, asparaginase, tamoxifen, flutamide, and taxol. Other preferred biologic agents include sense and/or antisense RNA or DNA sequences derived from specific tumor promoter or suppressor genes, such as, for example, the p53 and TGF gene families, signal transduction protein family members such as, for example, ras and myc, and growth factor receptor kinases such as, for

example flt2 and flk1, Tai1, Tai2, and neuropholin, and other genes implicated in neoplastic disease and other diseases driven by pathologic angiogenesis.

In another embodiment, GBS toxin receptor polypeptide or fragment thereof can be administered to a subject as a decoy to reduce the amount of stimulation of the GBS toxin receptor present in afflicted tissues (e.g., tumor tissues), thereby reducing cellular responses leading to proliferation and migration of cells of the afflicted tissues. Preferably, the GBS toxin receptor polypeptide or fragment is administered in soluble form, even more preferably sans transmembrane domains.

10 PHARMACEUTICAL COMPOSITIONS

Polypeptides of the invention that comprise a domain essential for GBS toxin binding that have the desired characteristics for bioavailability, stability and other important parameters of pharmacokinetics *in vivo* can be used as a competitive inhibitor of GBS toxin binding for medical conditions, such as, for example, early onset disease in the neonate, in which GBS toxin binding is undesirable. Appropriate polypeptides can include fragments having an amino acid sequence corresponding to a partial or full sequence of SEQ ID NO: 2 or SEQ ID NO: 4 or analogs thereof.

Compounds determined by assays using the polypeptides of the invention to bind and/or GBS toxin receptor and/or induce an inflammatory response, and that have the desired pharmacokinetic characteristics, can be used as treatments for medical conditions in which GBS toxin binding can be therapeutic, such as, for example, medical conditions involving pathologic or hypoxia-driven angiogenesis or neovascularization.

Pharmaceutical compositions of the invention include a pharmaceutically acceptable carrier that may contain a variety of components that provide a variety of functions, including regulation of drug concentration, regulation of solubility, chemical stabilization, regulation of viscosity, absorption enhancement, regulation of pH, and the like. For example, in water soluble formulations the pharmaceutical composition preferably includes a buffer such as a phosphate buffer, or other organic acid salt, preferably at a pH of between about 7 and 8. Other components may include antioxidants, such as ascorbic acid, hydrophilic polymers, such as, monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, dextrans, chelating agents, such as EDTA, and like components well

known to those in the pharmaceutical sciences, e.g. Remington's Pharmaceutical Science, latest edition (Mack Publishing Company, Easton, PA).

An effective amount of an active compound such as a GBS toxin receptor polypeptide, mimetic or analog, or GBS toxin mimetic or analog for particular applications depends on several factors, including the chemical nature of the polypeptide, mimetic or analog, the disorder being treated, the method of administration, and the like. Preferably, an effective amount will provide a concentration of polypeptide or mimetic of between about 0.0001 to 100 μM at the target GBS toxin receptor on a cell surface, more preferably less than 10 μM , with less than 1 μM being most preferred.

The active compound can be administered to a mammalian host in a variety of forms, i.e., they may be combined with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, elixirs, syrups, injectable or eye drop solutions, and the like depending on the chosen route of administration, e.g., orally or parenterally. Parenteral administration in this respect includes administration by the following routes: intravenous, intramuscular, subcutaneous, intraocular, intrasynovial, transepithelial (including transdermal, ophthalmic, sublingual and buccal), topical (including ophthalmic, dermal, ocular, rectal, nasal inhalation via insufflation and aerosol), and rectal systemic.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, it may be enclosed in hard or soft shell gelatin capsules, compressed into tablets, or incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2% to about 6% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 1 and 1000 mg of active compound.

Tablets, troches, pills, capsules and the like may also contain the following: a binder such as polyvinylpyrrolidone, gum tragacanth, acacia, sucrose, corn starch or gelatin; an excipient such as calcium phosphate, sodium citrate and calcium carbonate; a disintegrating agent such as corn starch, potato starch, tapioca starch, 5 certain complex silicates, alginic acid and the like; a lubricant such as sodium lauryl sulfate, talc and magnesium stearate; a sweetening agent such as sucrose, lactose or saccharin; or a flavoring agent such as peppermint, oil of wintergreen or cherry flavoring. Solid compositions of a similar type are also employed as fillers in soft and hard-filled gelatin capsules; preferred materials in this connection also include lactose 10 or milk sugar as well as high molecular weight polyethylene glycols. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the 15 active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye, flavoring such as cherry or orange flavor, emulsifying agents and/or suspending agents, as well as such diluents as water, ethanol, propylene glycol, glycerin and various combinations thereof. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in 20 the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

The active compound may also be administered parenterally or intraperitoneally. For purposes of parenteral administration, solutions in sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous 25 solutions of the corresponding water-soluble, alkali metal or alkaline-earth metal salts previously enumerated. Such aqueous solutions should be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. Solutions of the active compound as a free base or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as 30 hydroxypropylcellulose. A dispersion can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. In

this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying technique which yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

For purposes of topical administration, dilute sterile, aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the above parenteral solutions, are prepared in containers suitable for drop-wise administration to the eye. The compounds of this invention may be administered to a mammal alone or in combination with pharmaceutically acceptable carriers. As noted above, the relative proportions of active ingredient and carrier are determined by the solubility and

chemical nature of the compound, chosen route of administration, the particular compound chosen and the physiological characteristics of the particular patient under treatment.

5 **KITS**

Yet another aspect of the invention is a kit for use in carrying out any of the above methods. A preferred embodiment is a kit comprising a GBS toxin receptor or fragment thereof. Preferably, the receptor or fragment is immobilized. A preferred kit can be used for identifying a compound that binds to GBS toxin receptor, and

10 comprises at least one cell that expresses GBS toxin receptor.

Another embodiment is a kit for monitoring tumor growth or metastasis, comprising a reagent for detecting expression of a GBS toxin receptor. Examples of such reagents include, but are not limited to, polynucleotide probes that hybridize to a GBS toxin receptor nucleic acid sequence and compounds that bind to a GBS toxin

15 receptor, such as, for example, an antibody that specifically recognizes GBS toxin receptor, a GBS toxin, a GBS toxin mimetic, or other compounds identified by the screening methods described above.

A third embodiment is a kit for purifying a compound that binds a GBS toxin receptor, comprising a GBS toxin receptor or fragment thereof that binds the compound. Preferred compounds include GBS toxin, GBS toxin mimetics, antibodies that specifically bind GBS toxin receptor, and other compounds identified by the screening methods described above.

Additional kit components can include, but are not limited to, additional reagents required for detection, a reference standard(s), instructions for use, and the like. Suitable reference standards include positive controls, negative controls, photographs of such controls, tabulated or graphed data of such controls, and the like. The kits may further comprise instructions for carrying out the methods described above, preferably printed instructions.

EXAMPLES

EXAMPLE 1 - CLONING SHEEP GBS TOXIN RECEPTOR

Primary culture of sheep lung endothelial cells

Small pieces of primary lung tissues from a 7-week old sheep are cut into small pieces in Hank's balanced salt solution (HBSS) containing 10 mM HEPES buffer (Life Technology), 1% penicillin/streptomycin and 0.1% gentamycin, and are cultured in sheep lung complete medium (Life Technology) at 37°C. After one week of the culture, clones of sheep lung endothelial cells are identified by Cobblestone morphology and harvested into 24-well tissue culture plates (Falcon) using cloning rings. When the cells are confluent, they are detached by pancreatin and transferred to a 60-mm tissue culture Petri dish or a T-25 tissue culture flask (Falcon). When they are confluent again, they are split and cultured into a few 100-mm tissue culture plates (Falcon). Each split is considered to be one passage. The same procedure is repeated until enough cells ($\sim 10^8$) are obtained for isolation of mRNA.

Isolation of mRNA and construction of cDNA library

Poly(A)⁺ RNA is isolated from 9.2×10^7 sheep lung endothelial cells (passage 8 and 9) by a standard method (Pharmacia). A total of 16 μ g poly(A)⁺ RNA is acceptable amount obtained. 2.5 μ g mRNA can be used to construct a cDNA library. Poly(A)⁺ RNA is oligo(dT)-primed (with *Not* I restriction site) and converted into double-stranded cDNA. After adding a *Bst*XI/*Eco*R I adaptor, the cDNA is unidirectionally cloned into the *Bst*XI and *Not* I sites of pCDNA3.1(+) (Invitrogen).

E. coli Top10F' (Invitrogen) is used as a host strain for amplification. 5.38×10^6 primary clones are an acceptable number generated. The library is amplified by plating cells onto fifty large LB agar plates containing ampicillin (100 $\mu\text{g/ml}$). The plates are scraped and aliquoted so that each aliquot represents 10 plates. DNA is
5 purified by Qiagen Max columns (Qiagen).

Screening of cDNA library for a gene encoding GBS toxin receptor

To screen a cDNA library for a gene encoding GBS toxin receptor gene, a unique colorimetric method is used. Five μg plasmid DNA from each pool of cDNA library is used to transfect COS7 cells. The transfected cells are cultured in
10 four to eight 96-well tissue culture plates (Falcon) for transient expression. Each well contains about 20,000 transfected cells in DMEM medium (Life Technology). COS7 cells transfected with pCDNA3.1(+) are used as a control. After 3 days expression, the medium is carefully removed. Each well is rinsed 3 times with HPSS buffer containing Mg^{2+} and Ca^{2+} (wash buffer) (Life Technology).

15 The cells are then incubated with biotinylated toxin (50 μl per well; 1 to 1.5 $\mu\text{g/ml}$) at room temperature for 1 h. After the hour incubation, the biotinylated toxin is discarded and the wells are rinsed 3 times with the wash buffer. The cells are incubated with streptavidin- β -gal solution and each well is rinsed 3 times with the wash buffer. The cells are then incubated with PNPG (50 μl per well; 1 mg/ml in
20 substrate buffer) at 37°C. Absorbance at 405 nm is measured by an ELISA reader at 1 and 20 h, respectively. The cells which give the highest OD are harvested. Plasmid DNA is isolated by Hirt extraction. Plasmid DNA is amplified in *E. coli* to have enough DNA for the next transfection (enrichment).

Enrichment is done 8 times by this colorimetric method. The number
25 of the transfected cells loaded into each well is gradually decreased in the last few enrichments and untransfected cells are added to each well to give a total number of 20,000 cells per well for the cells to be confluent and to reduce background after 3 days' expression. At the last enrichment, each well has only 1 to 10 transfected cells. Cells giving the highest OD are harvested. DNA is isolated and amplified in *E. coli*.

30 A number of isolated clones are individually assayed by this colorimetric method. The clones which showed higher binding to CM101 are sequenced.

Sequence analysis

DNA sequence analysis of clone pFU102, which has a 2.1kb insert, revealed a sequence encoding a partial integral glycoprotein. N-terminal sequence was obtained by 5'RACE method (Life Technology) and a full-length gene is designated as SP55. Triple ligation yielded pCD55, which contains an entire coding region of SP55.

mRNA for the SP55 has 2844 nucleotides, encoding a protein of 495 amino acids with a predicated mass of 55 KDa, SP55. Analysis by the method of Klein et al. (Klein et al., *Biochim Biophys Acta*, 815:468-476 (1985)) classifies SP55 as an integral protein with seven transmembrane segments. SP55 has both N-glycosylation and kinase phosphorylation sites. A Swiss-Prot. search of SP55 did not reveal any high homology to known human proteins. However, SP55 has some identity (~ 30%) to renal sodium-dependent phosphate transporters from human, rabbit, mouse and rat. In addition, SP55 has some identity (~ 30 to 39%) to hypothetical proteins (HYP50 and HYP63) from *C. elegans*.

EXAMPLE 2 - CLONING HUMAN GBS TOXIN RECEPTOR

The sheep GBS toxin receptor sequence shares about 37% identity with HYP50 and about 33% identity to HYP63, two hypothetical proteins from *C. elegans*. In the regions corresponding to amino acid residues 180-186 and 443-449 of SEQ ID No. 2, five amino acids within a seven amino acid stretch are absolutely conserved among the three proteins.

A first degenerate oligonucleotide, CMR3-S: 5'-CGGGATCCCGCCNGCNATGCAYRSHRTSTGG-3' (SEQ ID No. 5), was designed to include all possible codons encoding the amino acid sequences of SP55, HYP50, and HYP63 in the 180-186 region. A second degenerate oligonucleotide, CMR4-AS2: 5'-GGAATTCCDGGDGCRATKTCNARRTRRTT-3' (SEQ ID No. 6), was designed to include the complementary sequences of all possible codons encoding the amino acid sequences of SP55, HYP50, and HYP63 in the 443-449 region.

Polymerase chain reaction (PCR) was conducted using these oligonucleotides and a human embryo lung cDNA library as a template. The reaction yielded three overlapping sequences approximately 400 bp in size, which encompass part of the nucleic acid sequence of SEQ ID No. 3. These sequences were then used as probes to clone the remainder of the gene, referred to herein and HP59 (SEQ ID NO: 7).

EXAMPLE 3 - PREPARATION OF ANTIBODIES AGAINST GBS TOXIN RECEPTOR

Rabbits are immunized with the synthetic peptides shown in Table 8. A-1 mg/ml solution of peptide plus KLH in 0.01M phosphate buffer is prepared. For the first immunization, 200 µg of peptide plus KLH (200 µl) and an equal volume of Freund's complete adjuvant, emulsified well before injection, is injected into 3-4 spots on the dorsal surface about the neck and shoulders of a rabbit. After two weeks, the second immunization (boost) is given at the same concentration of immunogen, but emulsified in Freund's incomplete adjuvant. The boost is delivered in the same region of the body. After another two weeks, blood is collected and assayed by ELISA for response against the peptide without KLH. Further boosts are given to improve antibody titer, if necessary.

Table 8**Immunogenic Peptides**

<u>Peptide</u>	<u>Amino Acid Sequence</u>	<u>Size</u>	<u>SEQ ID Ref.</u>
p56a	APSDGEEGSDRTPLLQRAPRAEPAPVC	27 aa	residues 8-35 of SEQ ID NO:4
p55a	LAPSDGEEGSDRTPL	15 aa	residues 7-22 of SEQ ID NO: 4
p57a	NTTAKDNRTSYECA	14 aa	residues 71-84 of SEQ ID NO: 4

Peptide p55 is a fragment of an extracellular domain of GBS toxin receptor. Peptide p57a is a fragment of an intracellular domain of GBS toxin receptor. Animals immunized with these peptides produce polyclonal antibodies Pab55 and Pab57, respectively.

EXAMPLE 4 - DETECTION OF GBS TOXIN RECEPTOR EXPRESSION IN TUMOR CELLS

This example shows that GBS toxin receptor can be detected in tumor cells. Immunohistochemistry is performed on paired human and mouse tissues of normal or tumor origin, using rabbit polyclonal antibodies Pab 55 and Pab 57.

Mouse and human tumor tissues are fixed in 10% neutral formalin. The tissues are then dehydrated, paraffin embedded and 10-20 x 8-micron sections are cut for immunohistochemical staining.

- Immunohistochemical analysis is performed with the automated Ventana Immunohistochemical Stainer according to the manufacturer's suggested protocol (Ventana, Tucson, Arizona). Sections are deparaffinated with xylene. The prepared sections are then treated with 1% hydrogen peroxide prepared in 30% aqueous methanol for 20 minutes at room temperature to quench endogenous peroxidase activity. The slides are then washed with PBS, blocked with 5% BSA and 5% goat serum in PBS, washed again and then incubated for 30 minutes at 37°C with the appropriate diluted (1:100) antibody. Horseradish peroxidase-labeled goat anti-rabbit IgG is used as a secondary antibody. For visualization, the sections are incubated with DAB/H₂O₂. The sections are finally incubated with a copper enhancer (Ventana) for 4 minutes, washed, counterstained with hematoxylin, and mounted in toluene-minus mounting medium. Photographic documentation is performed and images are stored for later review and analysis. The results are summarized in Table 9. The numbers refer to glass slides.

TABLE 9

Immunohistochemistry of tumor and normal tissues

(diff. = differentiated)

Human tissues:

	Antibody	Magnification	Signal
1. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 55	400x	+
2. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 55	400x	+
3. Normal ovary (96-08ZO08) control tissue	Pab 55	400x	-
4. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 57	400x	+
5. Ovary tumor (95-02VO 16) high grade papillary carcinoma	Pab 57	400x	+
6. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 57	400x	+
7. Normal ovary 96-08ZO08) control	Pab 57	400x	-
8. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 55	400x	+
9. Normal colon 9708VO08) control	Pab 55	400x	-
10. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 57	400x	+

11. Colon cancer 95-14664) poorly diff. Pab 57	400x	+
Adenocarcinoma		
12. Normal colon 9708VO08) control Pab 57	400x	-
13. Female breast cancer (97-IOV03a) Pab 55	400x	+
Invasive mammary carcinoma		
14. Male breast cancer (no code) Pab 55	400x	+
mammary carcinoma		
15. Normal female breast 97-12VO20-3) Pab 55	400x	-
control		
16. Female breast cancer 97-IOV03a) Pab 57	400x	+
Invasive mammary carcinoma		
17. Male breast cancer (no code) Pab 57	400x	+
mammary carcinoma		
18. Normal female breast (97-12VO20-3) Pab 57	400x	-
control		
19. Lung cancer (97- 1 OV022-5) poorly Pab 55	400x	+
diff. NOJ-small cell carcinoma		
20. Normal lung (98-0 1 VO 11) control Pab 55		-
21. Lung cancer (97-10VO22-5) poorly Pab 57	400x	+
diff. NOJ-small cell carcinoma		
22. Lung cancer (97-10VO22-5) poorly Pab 57	400x	+
diff. NOJ-small cell carcinoma		
23. Normal lung (98-0 1 VO 11) control Pab 57		-

Mouse Tissues:

	Antibody	Magnification	Signal
24. Madison Lung Tumor (MLT) untreated with CM 101	Pab 55		+
25. MLT untreated with CM 101	Pab 55		+
26. Normal mouse lung	Pab 55		-
27. MLT untreated with CM 101	Pab 57		+
28. Normal mouse lung	Pab 57		-

The Pab 55 antibody stains the cells lining a blood vessel in a human ovary cancer tissue section, but such staining is not apparent in cells of normal human ovary tissue (see FIG. 2A and 2B, respectively). Similar results are obtained with the Pab 57 antibody (see FIG. 3A and 3B). As shown in the above table and in FIGS. 2A-3B, antibodies raised to GBS toxin receptor fragments specifically bound to tumor tissues but not normal tissues, suggesting that GBS toxin receptor is expressed in tumor cells but not normal cells.

10 EXAMPLE 5— DETECTION OF GBS TOXIN RECEPTOR EXPRESSION IN MICE AFFLICTED WITH RHEUMATOID ARTHRITIS

This example shows that GBS toxin receptor can be detected in cells from a mammalian model for rheumatoid arthritis (RA). Mice with collagen-induced arthritis were treated with CM101 or carrier. CM101 reversed the inflammatory

damage and inhibited pannus formation. Mouse #8 and #15, which were treated with CM101, and two control mice (not treated with CM101) were sacrificed for immunohistochemistry.

5

TABLE 10
Immunohistochemistry of Rheumatoid Arthritic Mice

29. No CM 101	Pab 55	+
30. MOUSE 8 - 5' (vessel)	Pab 55	+
31. No CM 101	Pab 57	+
32. MOUSE 15 - 5' (vessel)	Pab 57	+
33. MOUSE 8 - 5' (between joint)	Pab 57	+
34. MOUSE 15 - 5'	Pab 57	+
35. No CM 101 (marrow)	Pab 57	+
36. MOUSE 15 - 5' (marrow)	Pab 57	+

As shown above Pab55 and Pab57 specifically bound to pathologic neovasculature in the pannus, suggesting that GBS toxin receptor is expressed in mice afflicted with rheumatoid arthritis. No binding of CM101 was observed in the normal neovasculature in the growth plate of the joints of the arthritic mice.

10

EXAMPLE 6 – TARGETED DELIVERY OF A CHIMERIC COMPOUND TO TISSUES EXPRESSING GBS TOXIN RECEPTOR

This example shows the targeted delivery of a chimeric compound to tissues expressing GBS toxin. The chimeric compound is a CM101-biotin conjugate. Mice with Madison Lung Tumors (MLT) are infused intravenously (i.v.) with biotinylated CM 101.

15

CM101 has been reacted with hydrazinylated biotin to form the biotin hydrazone at the reducing end of the polysaccharide CM101. Briefly, 25 micrograms of lyophilized CM101 is dissolved in 250 μ l labeling buffer at 100 mM sodium acetate, 0.02% sodium azide. Aqueous meta-periodate (125 μ l of 30 mM) is added and the oxidation is allowed to proceed in the dark for 30 minutes at room temperature. The reaction is terminated by adding 80 mM Na_2SO_3 to the solution. The resultant aldehydes are reacted with 125 μ l of 5 mM NHS-LC-Biotin (MW 556.58) for a 1 hour incubation at room temperature to form biotinylated CM101. Excess biotin is removed by dialysis against 1 liter of PBS at 4°C four times. The

20

25

product is purified by gel filtration on an Ultrahydrogel 1000 HPLC. lyophilized and stored at -70°C until use.

5 Tissues are recovered 5 min post infusion with CM101 and subjected to immunohistochemistry. Tumor and normal mouse tissue sections are analyzed for CM 101 binding by both mouse anti-CM101 mAb (7A3), followed by secondary mAb-HRP conjugate (referred to in FIG. 4B as MLT CM101-Biot.5' + McAb), or with avidin (which specifically binds biotin) conjugated with HRP (referred to in FIG. 4A as MLT CM101-Biot.5' + Strep.HRP).

10 FIGS. 4A-4C depict different sections taken from the same tumor and include a longitudinal view of the same blood vessel approximately in the center of the figures. The dark staining in FIG. 4A shows the localization of the biotin component in the cells lining the blood vessel. Similarly, FIG. 4B depicts the localization of the CM101 component in the cells lining the blood vessel. FIG. 4C is a negative control that was not exposed to CM101. The analysis clearly shows that 7A3 and avidin bind
15 to the same blood vessels in tumor tissue. Thus, biotin has been delivered to the blood vessel of the tumor tissue by virtue of its physical association with a compound (CM101) that binds the GBS toxin receptor.

These studies show that chimeric compounds can be delivered to tissues undergoing pathologic and/or hypoxia-driven angiogenesis or neovascularization. As
20 part of a chimeric compound, cytotoxic molecules can be directed to such tissues, e.g., tumor tissue. The cytotoxic molecule can be coupled directly to a molecule that binds GBS toxin receptor, e.g., GBS toxin. Alternatively, the molecule that binds GBS toxin receptor can be coupled to biotin and the cytotoxic molecule can be coupled to avidin.

EXAMPLE 7 – ENHANCED SENSITIVITY TO GBS-TOXIN-DEPENDENT CYTOTOXICITY OF CELLS EXPRESSING GBS TOXIN RECEPTOR

This example shows the enhanced sensitivity to GBS-toxin-dependent cytotoxicity of cells transfected with the GBS toxin receptor, relative to control cells.

- 5 Without being bound to a particular theory, the inventors believe that complement binds GBS toxin bound to the GBS toxin receptor on a cell, thereby targeting the cell for killing by white blood cells (WBC).

- Human bladder carcinoma cells (ECV cells), are stable transfected with the human GBS toxin receptor gene. The resultant cell line is ECV711. Cells stable
10 transfected with vector alone as referred to as V23. ECV 711 and V23 are seeded in 96-well plates at 5,000 cells/well.

- White blood cells are collected from healthy human donors as follows. Blood is collected by standard phlebotomy procedures into heparinized tubes (30 U/ml) and centrifuged at 2000 rpm for 20 min. The interface is carefully transferred to a new
15 tube and washed twice by centrifugation with medium (RPMI-1640). Cells are resuspended in RPMI-1640 supplemented with 5% fetal bovine serum (FBS) and Interferon-gamma (IFN) at 100 U/ml, and incubated overnight in a 37°C, 5%CO₂ incubator. The cells are then resuspended in fresh medium with 5% FBS.

- 5,000 cells of the WBC preparation are added to each well containing the
20 transfected cells. CM101 is added to a final concentration of 1µg/ml to the wells together with human serum from matching human donors. The cells are incubated 6 hours at 37 ° C.

- Cytotoxicity is assayed by measuring lactate dehydrogenase (LDH) using the Promega's CytoTox 96 Non-Radioactive Assay kit (Nachlas et al. (1960) *Anal.*
25 *Biochem* 1, 317; Korzeniewski et al. (1983) *J. Immunol. Methods* 64, 313; Decker et al. *J. Immunol. Methods* 115, 61; Brander et al. (1993) *Eur. J. Immunology* 23, 3217; Behl et al. (1994) *Cell* 77, 817; Lappalainen et al. (1994) *Pharm. Research* 11, 1127; Allen et al. (1994) *Promega Notes* 45, 7; Sinensky et al. (1995) *Toxicol. Letters* 75, 02; Moravec (1994) *Promega Notes* 45, 11). Percent cytotoxicity is calculated as
30 recommended by the manufacturer's instructions. The results are shown in Table 11.

Table 11

Cytotoxicity	ECV 711	V 23
WBC, IFN, C3, -CM101	29.1%	27.5%
WBC, IFN, C3, +CM101	40.45%	22.46%

There is an increase in cytotoxicity of 39% when the ECV 711 cells are incubated with CM101, WBC and human serum (source of C3) compared to cells
5 incubated without CM101. Control cells transfected with vector alone, V23, do not show a CM101 dependent increase in cytotoxicity.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication
or patent application was specifically and individually indicated to be incorporated by
10 reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide at least 10 bases in length comprising a nucleic acid sequence encoding, or complementary to a nucleic acid sequence encoding, a mammalian receptor for group B β -hemolytic Streptococcus toxin (GBS toxin receptor), or a polypeptide fragment thereof.
5
2. The polynucleotide of Claim 1, wherein the nucleic acid sequence comprises SEQ ID NO: 9.
3. The polynucleotide of Claim 1, wherein the nucleic acid sequence has 100% identity to a nucleic acid sequence selected from the group consisting of
10 residues 61 to 1542 of SEQ ID NO: 1, residues 266 to 1870 of SEQ ID NO: 7, and residues 87 to 1568 of SEQ ID NO: 3.
4. The polynucleotide of Claim 1, wherein the polynucleotide is hybridizable under high stringency conditions to the nucleic acid sequence of SEQ ID NO: 7.
5. A vector comprising the polynucleotide of Claim 1.
- 15 6. A host cell transformed with the vector of Claim 5.
7. A process for producing a mammalian GBS toxin receptor or fragment thereof, comprising culturing the host cell of Claim 6 in a suitable culture medium.
8. An isolated polypeptide comprising a mammalian GBS toxin receptor or fragment thereof.
- 20 9. The polypeptide of Claim 8, wherein the receptor has at least about 86% identity to the corresponding amino acid sequence of SEQ ID NO: 2.
10. The polypeptide of Claim 8, wherein the receptor or fragment has 100% identity to the corresponding region of the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 8.
- 25 11. The polypeptide of Claim 8, wherein the polypeptide is encoded by a nucleic acid sequence hybridizable under high stringency conditions to a nucleic acid sequence selected from the group consisting of:

- a) nucleotides 61 to 1542 of SEQ ID NO: 1, and
- b) nucleotides 87 to 1568 of SEQ ID NO: 3.

12. An isolated polypeptide comprising an amino acid sequence that differs from an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:8 at no more than about 20% of the amino acid residues.

13. The isolated polypeptide of Claim 12, wherein the amino acid sequence of said isolated polypeptide differs from the amino acid sequence selected from said group by one amino acid residue.

14. The isolated polypeptide of Claim 12, wherein the different amino acid residues are conservative substitutions of the corresponding residues of the amino acid sequence selected from said group.

15. An isolated polypeptide comprising an amino acid sequence of the formula:

AA1-AA_n-AA_m

wherein:

AA1 is absent or is M;

AA_n is a contiguous chain of 0 to 100 amino acids, preferably of 0 or 41 amino acids, even more preferably of residues 2-42 of SEQ ID NO:8; and

AA_m is a contiguous chain of 494 amino acids comprising AA43 through AA536, wherein:

(1) each of AA43, AA47, AA51, AA52, AA57, AA58, AA65, AA66, AA72, AA85, AA87, AA93, AA94, AA96, AA115, AA116, AA122, AA123, AA125, AA134, AA143, AA173, AA174, AA178, AA185, AA186, AA189, AA190, AA196, AA200, AA204, AA206, AA207, AA220, AA253, AA260, AA276, AA277, AA280, AA283, AA287, AA294, AA295, AA298, AA300, AA301, AA312, AA324, AA326, AA360, AA365, AA373, AA374, AA379, AA396, AA403, AA407, AA418, AA480, AA483, AA486, AA491, AA494, AA502, AA528, AA529, AA532 and AA536 is an amino acid residue corresponding to:

(a) residue 43, 47, 51, 52, 57, 58, 65, 66, 72, 85, 87, 93, 94, 96, 115, 116, 122, 123, 125, 134, 143, 173, 174, 178, 185, 186, 189, 190, 196, 200, 204, 206, 207, 220, 253, 260, 276, 277, 280, 283, 287, 294, 295, 298, 300, 301, 312, 324, 326, 360, 365, 373, 374, 379, 396, 403, 407, 418, 480, 483, 486, 491, 494, 502, 528, 529, 532 and 536, respectively, of SEQ ID NO:8;

(b) residue 2, 6, 10, 11, 16, 17, 24, 25, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283,

285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495, respectively of SEQ ID NO:4; or

(c) a conservative substitution thereof;

- (2) each of AA44-AA46, AA48-AA50, AA53-AA56, AA59-AA64, AA67-AA71, AA73-AA84, AA86, AA88-AA92, AA95, AA97-AA114, AA117-AA121, AA124, AA126-AA133, AA135-AA142, AA144-AA172, AA175-AA177, AA179-AA184, AA187-AA188, AA191-AA195, AA197-AA199, AA201-AA203, AA205, AA208-AA219, AA221-AA252, AA254-AA259, AA261-AA275, AA278-AA279, AA281-AA282, AA284-AA286, AA288-AA293, AA296-AA297, AA299, AA302-AA311, AA313-AA323, AA325, AA327-AA359, AA361-AA364, AA366-AA372, AA375-AA378, AA380-AA395, AA397-AA402, AA404-AA406, AA408-AA417, AA419-AA478, AA481-AA482, AA484-AA485, AA487-AA490, AA492-AA493, AA495-AA501, AA503-AA527, AA530-AA531 and AA533-AA535 is
- (a) residue 44-46, 48-50, 53-56, 59-64, 67-71, 73-84, 86, 88-92, 95, 97-114, 117-121, 124, 126-133, 135-142, 144-172, 175-177, 179-184, 187-188, 191-195, 197-199, 201-203, 205, 208-219, 221-252, 254-259, 261-275, 278-279, 281-282, 284-286, 288-293, 296-297, 299, 302-311, 313-323, 325, 327-359, 361-364, 366-372, 375-378, 380-395, 397-402, 404-406, 408-417, 419-478, 481-482, 484-485, 487-490, 492-493, 495-501, 503-527, 530-531 and 533-535, respectively, of SEQ ID NO:8; or

(b) a conservative substitutions thereof; and

(3) one or more of AA315 through AA367 are optionally absent.

16. An antibody that recognizes a mammalian GBS toxin receptor or fragment thereof.

17. An isolated complex comprising a GBS toxin bound to a mammalian GBS toxin receptor or fragment thereof.

18. A method of forming a complex comprising:

contacting a GBS toxin with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide, and allowing the complex to form.

19. A method for purifying a compound that binds a GBS toxin receptor, which method comprises:

providing a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that binds GBS toxin;

contacting said polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide; and

separating the bound compound from the remainder of the sample.

20. A method of determining the presence or absence of GBS toxin in a sample, which method comprises:

- 5 contacting the sample with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide, and determining whether specific binding has occurred.

21. A method for diagnosing early onset disease in a neonate comprising performing the method of Claim 20, wherein the sample is obtained from the neonate
10 and wherein presence of the GBS toxin is indicative of early onset disease.

22. A method for detecting pathologic vasculature in a mammalian tissue, which method comprises detecting the presence of a GBS toxin receptor.

23. A method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor, comprising:

- 15 combining a test compound with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, in a reaction mixture containing GBS toxin and under conditions that permit specific binding of the GBS toxin to the receptor or fragment, and determining the amount of inhibition by the compound of the binding
20 of the GBS toxin to the polypeptide.

24. An inhibitor of binding of a GBS toxin to a mammalian GBS toxin receptor.

25. A method for identifying a compound which specifically binds a mammalian GBS toxin receptor, comprising:

- 25 combining a test compound with a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that can bind GBS toxin, under conditions that allow specific binding to occur, and detecting a complex formed between said test compound and said

polypeptide.

26. A method for determining cytotoxicity of a test chimeric compound, which method comprises:

5 exposing a cell expressing, on the cell surface, a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, to a test chimeric compound comprising a cytotoxic agent coupled to said GBS toxin; and
detecting signs of cytotoxicity.

27. A chimeric compound comprising a cytotoxic agent covalently linked to a molecule that specifically binds a mammalian GBS toxin receptor.

10 28. A method for identifying an inhibitor of GBS toxin receptor, which method comprises:

incubating test cells in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, wherein the test cells express GBS toxin receptor or a
15 fragment thereof having GBS toxin receptor activity; and
comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being an
20 inhibitor of the GBS toxin receptor.

29. A method for identifying an inhibitor of endothelial cell proliferation or migration, which method comprises:

incubating test endothelial cells in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test
25 compound can proliferate or migrate, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity; and
comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration

in the presence of the test compound is indicative of the test compound being an inhibitor of the endothelial cell proliferation or migration.

30. A method for identifying a therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic angiogenesis or neovascularization, which method comprises:

incubating test cells in the presence and absence of a test compound, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity;

- comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being a candidate therapeutic compound for the treatment or prevention of the medical condition.

31. The method of Claim 30, wherein the medical condition is a cancerous tumor.

32. The method of Claim 30, wherein the medical condition is a reperfusion injury.

33. The method of Claim 30, wherein the medical condition is scarring during wound healing.

34. The method of Claim 30, wherein the medical condition is keloids.

35. The method of Claim 30, wherein the medical condition is a chronic inflammatory disease.

36. The method of Claim 30, wherein the medical condition is neural injury.

37. A method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor, comprising:

(a) simulating and selecting the most probable conformations of a mammalian GBS toxin receptor,

- (b) designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the polypeptide,
- (c) chemically synthesizing the analog, and
- (d) evaluating the bioactivity of the analog.

5 38. A method for identifying a compound which binds to a mammalian GBS toxin receptor, comprising:

- (a) simulating and selecting the most probable conformations of a mammalian GBS toxin receptor,
- (b) deducing the most probable binding domains of the polypeptide,
- 10 (c) designing a compound that would form the energetically most probable complexes with the polypeptide,
- (d) chemically synthesizing the compound, and
- (e) evaluating the bioactivity of the compound.

15 39. A method for the prevention or treatment of neonatal onset disease in a human neonate, comprising administering an inhibitor of binding of GBS toxin to a human GBS toxin receptor.

40. A method for inhibiting pathologic or hypoxia-driven endothelial cell proliferation or migration in a mammalian tissue, which method comprises specifically binding a molecule to a GBS toxin receptor present on the surface of at
20 least one cell in the tissue, the molecule being selected from the group consisting of:

 a compound that can evoke an inflammatory response when bound to a GBS toxin receptor in a mammal;

 a chimeric compound comprising a cytotoxic compound coupled to a compound that specifically binds the GBS toxin receptor;

25 an inhibitor of GBS toxin receptor phosphorylation; and
 an inhibitor of GBS toxin receptor activity.

41. A pharmaceutical composition comprising a pharmaceutically effective amount of a molecule selected from the group consisting of:

 a GBS toxin receptor or fragment thereof;

an inhibitor of a GBS toxin receptor; and
a chimeric compound comprising a cytotoxic agent coupled to a
compound that binds GBS toxin receptor,
and a pharmaceutically acceptable carrier.

5

42. A kit comprising a component selected from the group consisting of:

a GBS toxin receptor or fragment;

a reagent for detecting the presence of a GBS toxin receptor or
fragment; and

10

a reagent for detecting the presence of polynucleotide encoding the
GBS toxin receptor or fragment.

43. A molecule for use in a method of treatment of the human or animal body,
said molecule being selected from the group consisting of:

a GBS toxin receptor or fragment thereof for use in a method of
15 treatment of the human or animal body, said molecule being selected from the group
consisting of:

a GBS toxin receptor or fragment thereof;

an inhibitor of binding of GBS toxin to a GBS toxin receptor;

an inhibitor of a GBS toxin receptor; and

20

a chimeric compound comprising a cytotoxic agent coupled to a
compound that binds GBS toxin receptor.

44. Use of an inhibitor of a GBS toxin receptor, or of an inhibitor of binding of
GBS toxin to a GBS toxin receptor, for the manufacture of a medicament for the
treatment of a medical condition characterized by pathologic or hypoxia-driven
25 angiogenesis or neovascularization.

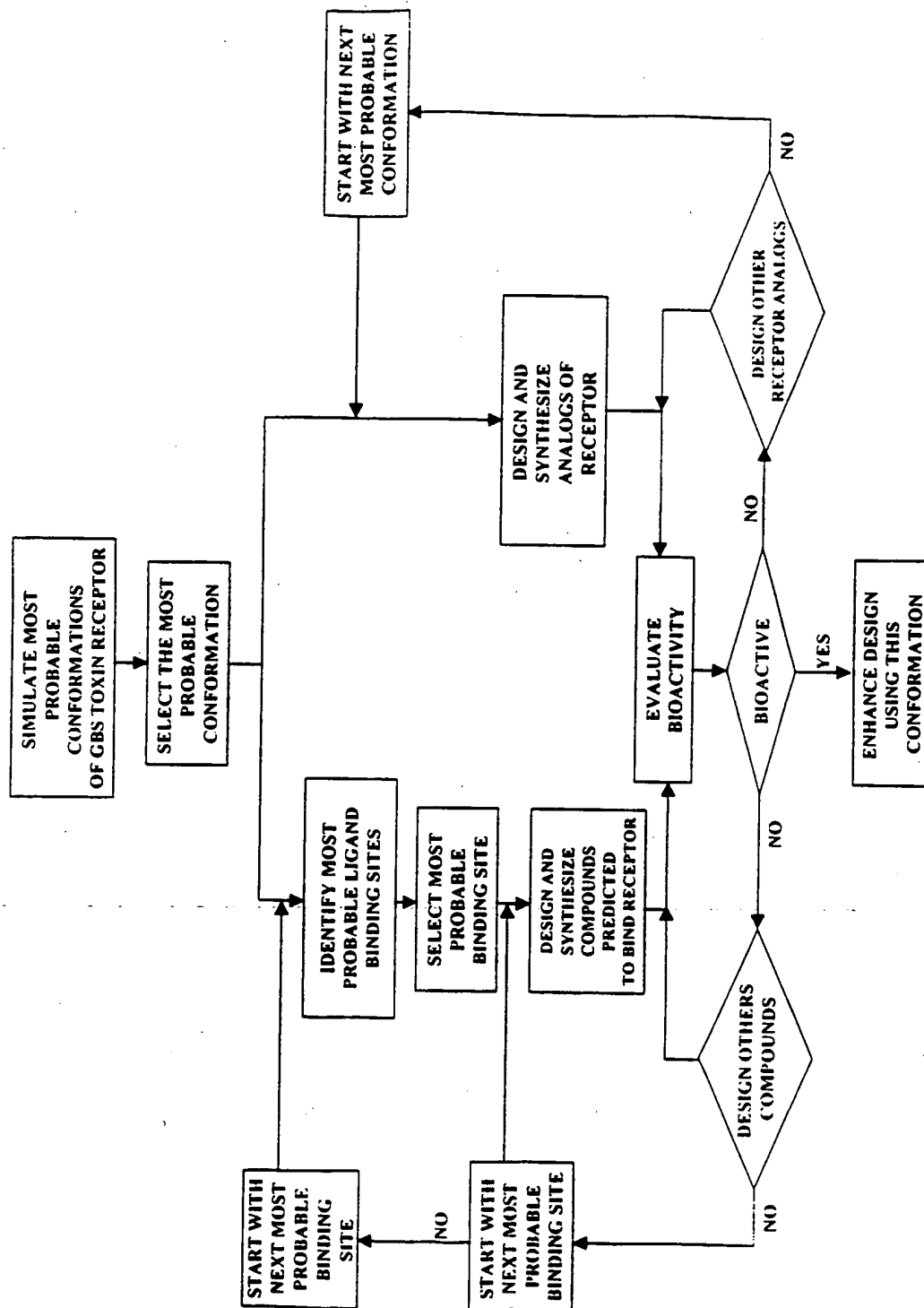


FIG. 1



**Normal Human
Ovary+Pab 55**

FIG. 2B



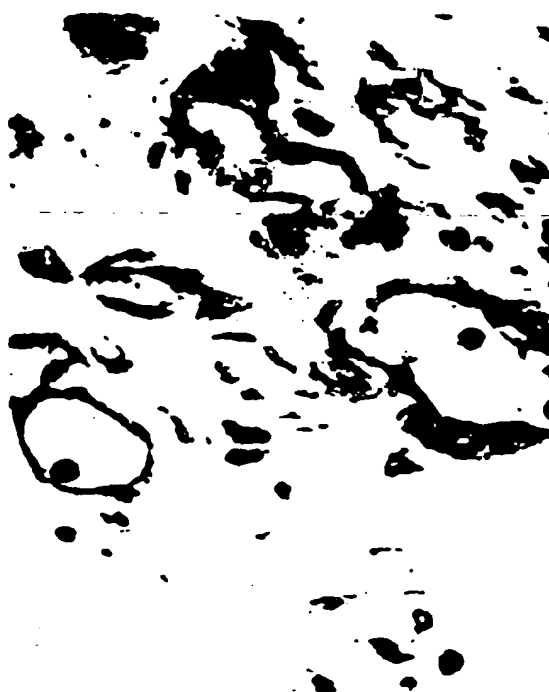
**Human Ovary
Cancer+Pab 55**

FIG. 2A



**Normal Human
Ovary+Pab 57**

FIG. 3B



**Human Ovary
Cancer+Pab 57**

FIG. 3A



**MLT CM101-Biot.5'
+McAb**

FIG. 4B



**MLT CM101-Biot.5'
+Strep.HRP**

FIG. 4A



MLT-PBS 5' + Streptavidin-HRP

FIG. 4C

SEQUENCE LISTING

<110> Hellergvist, Carl
Fu, Changlin

<120> GBS Toxin Receptor

<130> CARB-008/01WO

<140>

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<150> 60-093,843

<151> 1998-07-22

<160> 12

<170> PatentIn Ver. 2.0

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<211> 2602

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<222> (58)..(1542)

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atg agg tct ccg gtt cga gac ctg gcc cgg aac gat ggc gag gag agc      105
Met Arg Ser Pro Val Arg Asp Leu Ala Arg Asn Asp Gly Glu Glu Ser
  1             5             10             15

acg gac cgc acg cct ctt cta ccg ggc gcc cca cgg gcc gaa gcc gct      153
Thr Asp Arg Thr Pro Leu Leu Pro Gly Ala Pro Arg Ala Glu Ala Ala
      20             25             30

cca gtg tgc tgc tct gct cgt tac aac tta gca att ttg gcc ttt ttt      201
Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Ile Leu Ala Phe Phe
      35             40             45

ggg ttc ttc att gtg tat gca tta cgt gtg aat ctg agt gtt gcg tta      249
Gly Phe Phe Ile Val Tyr Ala Leu Arg Val Asn Leu Ser Val Ala Leu
      50             55             60

gtg gat atg gta gat tca aat aca act tta gaa gat aat aga act tcc      297

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Val	Asp	Met	Val	Asp	Ser	Asn	Thr	Thr	Leu	Glu	Asp	Asn	Arg	Thr	Ser	
65						70					75				80	
aag	gcg	tgt	cca	gag	cat	tct	gct	ccc	ata	aaa	gtt	cat	cat	aat	caa	345
Lys	Ala	Cys	Pro	Glu	His	Ser	Ala	Pro	Ile	Lys	Val	His	His	Asn	Gln	
				85					90					95		
acg	ggt	aag	aag	tac	caa	tgg	gat	gca	gaa	act	caa	gga	tgg	att	ctc	393
Thr	Gly	Lys	Lys	Tyr	Gln	Trp	Asp	Ala	Glu	Thr	Gln	Gly	Trp	Ile	Leu	
				100				105					110			
ggt	tcc	ttt	ttt	tat	ggc	tac	atc	atc	aca	cag	att	cct	gga	gga	tat	441
Gly	Ser	Phe	Phe	Tyr	Gly	Tyr	Ile	Ile	Thr	Gln	Ile	Pro	Gly	Gly	Tyr	
		115					120						125			
gtt	gcc	agc	aaa	ata	ggg	ggg	aaa	atg	ctg	cta	gga	ttt	ggg	atc	ctt	489
Val	Ala	Ser	Lys	Ile	Gly	Gly	Lys	Met	Leu	Leu	Gly	Phe	Gly	Ile	Leu	
		130				135						140				
ggc	act	gct	gtc	ctc	acc	ctg	ttc	act	ccc	att	gct	gca	gat	tta	gga	537
Gly	Thr	Ala	Val	Leu	Thr	Leu	Phe	Thr	Pro	Ile	Ala	Ala	Asp	Leu	Gly	
145					150					155				160		
gtt	gga	cca	ctc	att	gta	ctc	aga	gca	cta	gaa	gga	cta	gga	gag	ggt	585
Val	Gly	Pro	Leu	Ile	Val	Leu	Arg	Ala	Leu	Glu	Gly	Leu	Gly	Glu	Gly	
			165					170					175			
gtt	aca	ttt	cca	gcc	atg	cat	gcc	atg	tgg	tct	tct	tgg	gct	ccc	cct	633
Val	Thr	Phe	Pro	Ala	Met	His	Ala	Met	Trp	Ser	Ser	Trp	Ala	Pro	Pro	
			180					185					190			
ctt	gaa	aga	agc	aaa	ctt	ctt	agc	att	tcg	tat	gca	gga	gca	cag	ctt	681
Leu	Glu	Arg	Ser	Lys	Leu	Leu	Ser	Ile	Ser	Tyr	Ala	Gly	Ala	Gln	Leu	
		195					200					205				
ggg	aca	gta	att	tct	ctt	cct	ctt	tct	gga	ata	att	tgc	tac	tat	atg	729
Gly	Thr	Val	Ile	Ser	Leu	Pro	Leu	Ser	Gly	Ile	Ile	Cys	Tyr	Tyr	Met	
		210					215					220				
aat	tgg	act	tat	gtc	ttc	tac	ttt	ttt	ggt	act	att	gga	ata	ttt	tgg	777
Asn	Trp	Thr	Tyr	Val	Phe	Tyr	Phe	Phe	Gly	Thr	Ile	Gly	Ile	Phe	Trp	
225					230					235				240		
ttt	ctt	ttg	tgg	atc	tgg	tta	gtt	agt	gac	aca	cca	caa	aaa	cac	aag	825
Phe	Leu	Leu	Trp	Ile	Trp	Leu	Val	Ser	Asp	Thr	Pro	Gln	Lys	His	Lys	
				245					250				255			
aga	att	tcc	cat	tat	gaa	aag	gaa	tac	att	ctt	tca	tca	tta	aga	aat	873

Arg Ile Ser His Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Arg Asn
 260 265 270

cag ctt tct tca cag aag tca gtg ccg tgg gta ccc att tta aaa tcc 921
 Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Val Pro Ile Leu Lys Ser
 275 280 285

ctg cca ctt tgg gct atc gta gtt gca cac ttt tct tac aac tgg act 969
 Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr
 290 295 300

ttt tat act tta ttg aca tta ttg cct act tat atg aag gag atc cta 1017
 Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Ile Leu
 305 310 315 320

agg ttc aat gtt caa gag aat ggg ttt tta tct tca ttg cct tat tta 1065
 Arg Phe Asn Val Gln Glu Asn Gly Phe Leu Ser Ser Leu Pro Tyr Leu
 325 330 335

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 Gly Ser Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu
 340 345 350

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 Arg Ala Lys Trp Asn Phe Ser Thr Leu Cys Val Arg Arg Ile Phe Ser
 355 360 365

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 370 375 380

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 Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr
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 Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp
 405 410 415

att gct cct tcg tat gct ggt atc ctc ctg ggc atc aca aat aca ttt 1353
 Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe
 420 425 430

gcc act att cca gga atg gtt ggg ccc gtc att gct aaa agt ctg acc 1401
 Ala Thr Ile Pro Gly Met Val Gly Pro Val Ile Ala Lys Ser Leu Thr
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cct gat aac act gtt gga gaa tgg caa acc gtg ttc tat att gct gct 1449

Pro Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala
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gct att aat gtt ttt ggt gcc att ttc ttt aca cta ttc gcc aaa ggt 1497
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 agacctcaaa ctttagcatc tctgtggagc tgccatccac tgtataattt cgcctggcaa 2022
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 <213> Homo sapiens

<400> 2

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      35           40           45

Gly Phe Phe Ile Val Tyr Ala Leu Arg Val Asn Leu Ser Val Ala Leu
      50           55           60

Val Asp Met Val Asp Ser Asn Thr Thr Leu Glu Asp Asn Arg Thr Ser
      65           70           75           80

Lys Ala Cys Pro Glu His Ser Ala Pro Ile Lys Val His His Asn Gln
      85           90           95

Thr Gly Lys Lys Tyr Gln Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
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Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr
      115          120          125

Val Ala Ser Lys Ile Gly Gly Lys Met Leu Leu Gly Phe Gly Ile Leu
      130          135          140

Gly Thr Ala Val Leu Thr Leu Phe Thr Pro Ile Ala Ala Asp Leu Gly
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Val Gly Pro Leu Ile Val Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly
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Val Thr Phe Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro
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Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu
      195          200          205

Gly Thr Val Ile Ser Leu Pro Leu Ser Gly Ile Ile Cys Tyr Tyr Met
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Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Thr Ile Gly Ile Phe Trp
 225 230 235 240
 Phe Leu Leu Trp Ile Trp Leu Val Ser Asp Thr Pro Gln Lys His Lys
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 Arg Ile Ser His Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Arg Asn
 260 265 270
 Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Val Pro Ile Leu Lys Ser
 275 280 285
 Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr
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 Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Ile Leu
 305 310 315 320
 Arg Phe Asn Val Gln Glu Asn Gly Phe Leu Ser Ser Leu Pro Tyr Leu
 325 330 335
 Gly Ser Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu
 340 345 350
 Arg Ala Lys Trp Asn Phe Ser Thr Leu Cys Val Arg Arg Ile Phe Ser
 355 360 365
 Leu Ile Gly Met Ile Gly Pro Ala Val Phe Leu Val Ala Ala Gly Phe
 370 375 380
 Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr
 385 390 395 400
 Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp
 405 410 415
 Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe
 420 425 430
 Ala Thr Ile Pro Gly Met Val Gly Pro Val Ile Ala Lys Ser Leu Thr
 435 440 445
 Pro Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala
 450 455 460
 Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
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 485 490 495

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 <213> Ovis sp.

<220>
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 <222> (84)..(1568)

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 Met Lys Ser Pro Val Ser Asp Leu Ala Pro
 1 5 10
 agc gac ggc gag gag ggc tcg gac cgc aca ccg ctc ctg cag cgc gcc 161
 Ser Asp Gly Glu Glu Gly Ser Asp Arg Thr Pro Leu Leu Gln Arg Ala
 15 20 25
 ccg cgg gcg gaa ccc gct cca gta tgc tgc tct gct cgt tac aac cta 209
 Pro Arg Ala Glu Pro Ala Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu
 30 35 40
 gca ttt ttg tcc ttt ttt ggt ttc ttc gtt ctc tat tca tta cgg gtg 257
 Ala Phe Leu Ser Phe Phe Gly Phe Phe Val Leu Tyr Ser Leu Arg Val
 45 50 55
 aat ctg agc gtt gca cta gtg gac atg gtg gat tca aac aca act gcc 305
 Asn Leu Ser Val Ala Leu Val Asp Met Val Asp Ser Asn Thr Thr Ala
 60 65 70

 aaa gat aat aga acg tcc tac gag tgt gca gag cat tct gct ccc ata 353
 Lys Asp Asn Arg Thr Ser Tyr Glu Cys Ala Glu His Ser Ala Pro Ile
 75 80 85 90
 aaa gtt ctt cac aac caa acg ggt aaa aag tac cgg tgg gat gca gaa 401
 Lys Val Leu His Asn Gln Thr Gly Lys Lys Tyr Arg Trp Asp Ala Glu
 95 100 105
 act caa gga tgg att ctc gga tct ttt ttc tat ggc tac atc atc aca 449
 Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr
 110 115 120

caa att cct gga gga tat gtt gcc agc aga agt ggg ggg aag ctg ttg	497
Gln Ile Pro Gly Gly Tyr Val Ala Ser Arg Ser Gly Gly Lys Leu Leu	
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Leu Gly Phe Gly Ile Phe Ala Thr Ala Ile Phe Thr Leu Phe Thr Pro	
140 145 150	
ctc gct gca gat ttc gga gtc gga gcc ctt gtt gca ctc agg gca cta	593
Leu Ala Ala Asp Phe Gly Val Gly Ala Leu Val Ala Leu Arg Ala Leu	
155 160 165 170	
gaa ggg cta gga gag ggt gtc aca tat cca gcc atg cat gcc atg tgg	641
Glu Gly Leu Gly Glu Gly Val Thr Tyr Pro Ala Met His Ala Met Trp	
175 180 185	
tct tca tgg gct ccc cct ctt gaa aga agc aag ctt ctg agt att tca	689
Ser Ser Trp Ala Pro Pro Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser	
190 195 200	
tat gca gga gca caa ctt ggg aca gta gtt tct ctt cct ctt tct gga	737
Tyr Ala Gly Ala Gln Leu Gly Thr Val Val Ser Leu Pro Leu Ser Gly	
205 210 215	
gta att tgc tac tat atg aat tgg act tat gtc ttc tat ttc ttt ggc	785
Val Ile Cys Tyr Tyr Met Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly	
220 225 230	
att gtt gga atc atc tgg ttt att tta tgg atc tgc tta gtt agt gat	833
Ile Val Gly Ile Ile Trp Phe Ile Leu Trp Ile Cys Leu Val Ser Asp	
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Thr Pro Glu Thr His Lys Thr Ile Thr Pro Tyr Glu Lys Glu Tyr Ile	
255 260 265	
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Leu Ser Ser Leu Lys Asn Gln Leu Ser Ser Gln Lys Ser Val Pro Trp	
270 275 280	
ata cct atg ctg aaa tca ctg cca ctt tgg gct att gtc gtt gca cat	977
Ile Pro Met Leu Lys Ser Leu Pro Leu Trp Ala Ile Val Val Ala His	
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Phe Ser Tyr Asn Trp Thr Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr	
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Tyr Met Lys Glu Val Leu Arg Phe Asn Ile Gln Glu Asn Gly Phe Leu	
315 320 325 330	
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Ser Ala Val Pro Tyr Leu Gly Cys Trp Leu Cys Met Ile Leu Ser Gly	
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gtt cga aga gtt ttt agc ctt ata ggg atg att gga cct gcg ata ttc	1217
Val Arg Arg Val Phe Ser Leu Ile Gly Met Ile Gly Pro Ala Ile Phe	
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Leu Val Ala Ala Gly Phe Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala	
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Phe Leu Thr Ile Ser Thr Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe	
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Ser Ile Asn His Leu Asp Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu	
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Gly Ile Thr Asn Thr Phe Ala Thr Ile Pro Gly Met Ile Gly Pro Ile	
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Ile Ala Arg Ser Leu Thr Pro Glu Asn Thr Ile Gly Glu Trp Gln Thr	
445 450 455	
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Val Phe Cys Ile Ala Ala Ala Ile Asn Val Phe Gly Ala Ile Phe Phe	
460 465 470	
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Thr Leu Phe Ala Lys Gly Glu Val Gln Asn Trp Ala Ile Ser Asp His	
475 480 485 490	
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Gln Gly His Arg Asn	
495	

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<212> PRT

<213> Ovis sp.

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 35 40 45

Gly Phe Phe Val Leu Tyr Ser Leu Arg Val Asn Leu Ser Val Ala Leu
 50 55 60

Val Asp Met Val Asp Ser Asn Thr Thr Ala Lys Asp Asn Arg Thr Ser
 65 70 75 80

Tyr Glu Cys Ala Glu His Ser Ala Pro Ile Lys Val Leu His Asn Gln
 85 90 95

Thr Gly Lys Lys Tyr Arg Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
 100 105 110

Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr
 115 120 125

Val Ala Ser Arg Ser Gly Gly Lys Leu Leu Leu Gly Phe Gly Ile Phe
 130 135 140

Ala Thr Ala Ile Phe Thr Leu Phe Thr Pro Leu Ala Ala Asp Phe Gly
 145 150 155 160

Val Gly Ala Leu Val Ala Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly
 165 170 175

Val Thr Tyr Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro
 180 185 190

Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu
 195 200 205

Gly Thr Val Val Ser Leu Pro Leu Ser Gly Val Ile Cys Tyr Tyr Met
 210 215 220

Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Ile Val Gly Ile Ile Trp
 225 230 235 240

Phe Ile Leu Trp Ile Cys Leu Val Ser Asp Thr Pro Glu Thr His Lys
 245 250 255

Thr Ile Thr Pro Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Lys Asn
 260 265 270
 Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Ile Pro Met Leu Lys Ser
 275 280 285
 Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr
 290 295 300
 Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Val Leu
 305 310 315 320
 Arg Phe Asn Ile Gln Glu Asn Gly Phe Leu Ser Ala Val Pro Tyr Leu
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 Gly Cys Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu
 340 345 350
 Arg Ala Arg Trp Asn Phe Ser Thr Leu Trp Val Arg Arg Val Phe Ser
 355 360 365
 Leu Ile Gly Met Ile Gly Pro Ala Ile Phe Leu Val Ala Ala Gly Phe
 370 375 380
 Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr
 385 390 395 400
 Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp
 405 410 415
 Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe
 420 425 430
 Ala Thr Ile Pro Gly Met Ile Gly Pro Ile Ile Ala Arg Ser Leu Thr
 435 440 445
 Pro Glu Asn Thr Ile Gly Glu Trp Gln Thr Val Phe Cys Ile Ala Ala
 450 455 460
 Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
 465 470 475 480
 Glu Val Gln Asn Trp Ala Ile Ser Asp His Gln Gly His Arg Asn
 485 490 495

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<211> 31
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 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: PCR primer

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31

<210> 6
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: PCR primer

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29

<210> 7
 <211> 2930
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (263)..(1870)

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 gcgggagacg gtcgtccgaa caccggctcc ccggcatgcg tagaccggcg ggcggagcgg 180
 gctcactttg cgccaatect acgagaactc ccagaactcc gttccctag tccaacccaa 240
 gccagagttg cccacaccta ag atg gcg gcg ggg gcg atg aca ccg ccc cgc 292
 Met Ala Ala Gly Ala Met Thr Pro Pro Arg
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 Pro Val Gln Pro Ala Arg Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg
 15 20 25

tcc ctt ctc tgc cag gtg gcg agt aca cct gct cac gta ggc gtc atg	388
Ser Leu Leu Cys Gln Val Ala Ser Thr Pro Ala His Val Gly Val Met	
30 35 40	
agg tct ccg gtt cga gac ctg gcc cgg aac gat ggc gag gag agc acg	436
Arg Ser Pro Val Arg Asp Leu Ala Arg Asn Asp Gly Glu Ser Thr	
45 50 55	
gac cgc acg cct ctt cta ccg ggc gcc cca cgg gcc gaa gcc gct cca	484
Asp Arg Thr Pro Leu Leu Pro Gly Ala Pro Arg Ala Glu Ala Ala Pro	
60 65 70	
gtg tgc tgc tct gct cgt tac aac tta gca att ttg gcc ttt ttt ggt	532
Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Ile Leu Ala Phe Phe Gly	
75 80 85 90	
ttc ttc att gtg tat gca tta cgt gtg aat ctg agt gtt gcg tta gtg	580
Phe Phe Ile Val Tyr Ala Leu Arg Val Asn Leu Ser Val Ala Leu Val	
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gat atg gta gat tca aat aca act tta gaa gat aat aga act tcc aag	628
Asp Met Val Asp Ser Asn Thr Thr Leu Glu Asp Asn Arg Thr Ser Lys	
110 115 120	
gcg tgt cca gag cat tct gct ccc ata aaa gtt cat cat aat caa acg	676
Ala Cys Pro Glu His Ser Ala Pro Ile Lys Val His His Asn Gln Thr	
125 130 135	
ggg aag aag tac caa tgg gat gca gaa act caa gga tgg att ctc ggt	724
Gly Lys Lys Tyr Gln Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu Gly	
140 145 150	
tcc ttt ttt tat ggc tac atc atc aca cag att cct gga gga tat gtt	772
Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr Val	
155 160 165 170	
gcc agc aaa ata ggg ggg aaa atg ctg cta gga ttt ggg atc ctt ggc	820
Ala Ser Lys Ile Gly Gly Lys Met Leu Leu Gly Phe Gly Ile Leu Gly	
175 180 185	
act gct gtc ctc acc ctg ttc act ccc att gct gca gat tta gga gtt	868
Thr Ala Val Leu Thr Leu Phe Thr Pro Ile Ala Ala Asp Leu Gly Val	
190 195 200	
gga cca ctc att gta ctc aga gca cta gaa gga cta gga gag ggt gtt	916
Gly Pro Leu Ile Val Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly Val	
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 220 225 230

gaa aga agc aaa ctt ctt agc att tgc tat gca gga gca cag ctt ggg 1012
 Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu Gly
 235 240 245 250

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 Thr Val Ile Ser Leu Pro Leu Ser Gly Ile Ile Cys Tyr Tyr Met Asn
 255 260 265

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 Trp Thr Tyr Val Phe Tyr Phe Phe Gly Thr Ile Gly Ile Phe Trp Phe
 270 275 280

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 Leu Leu Trp Ile Trp Leu Val Ser Asp Thr Pro Gln Lys His Lys Arg
 285 290 295

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 Ile Ser His Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Arg Asn Gln
 300 305 310

ctt tct tca cag aag tca gtg ccg tgg gta ccc att tta aaa tcc ctg 1252
 Leu Ser Ser Gln Lys Ser Val Pro Trp Val Pro Ile Leu Lys Ser Leu
 315 320 325 330

cca ctt tgg gct atc gta gtt gca cac ttt tct tac aac tgg act ttt 1300
 Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr Phe
 335 340 345

tat act tta ttg aca tta ttg cct act tat atg aag gag atc cta agg 1348
 Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Ile Leu Arg
 350 355 360

ttc aat gtt caa gag aat ggg ttt tta tct tca ttg cct tat tta ggc 1396
 Phe Asn Val Gln Glu Asn Gly Phe Leu Ser Ser Leu Pro Tyr Leu Gly
 365 370 375

tct tgg tta tgt atg atc ctg tct ggt caa gct gct gac aat tta agg 1444
 Ser Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu Arg
 380 385 390

gca aaa tgg aat ttt tca act tta tgt gtt cgc aga att ttt agc ctt 1492
 Ala Lys Trp Asn Phe Ser Thr Leu Cys Val Arg Arg Ile Phe Ser Leu
 395 400 405 410

ata gga atg att gga cct gca gta ttc ctg gta gct gct ggc ttc att 1540
 Ile Gly Met Ile Gly Pro Ala Val Phe Leu Val Ala Ala Gly Phe Ile
 415 420 425

ggc tgt gat tat tct ttg gcc gtt gct ttc cta act ata tca aca aca 1588
 Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr Thr
 430 435 440

ctg gga ggc ttt tgc tct tct gga ttt agc atc aac cat ctg gat att 1636
 Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp Ile
 445 450 455

gct cct tcg tat gct ggt atc ctc ctg ggc atc aca aat aca ttt gcc 1684
 Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe Ala
 460 465 470

act att cca gga atg gtt ggg ccc gtc att gct aaa agt ctg acc cct 1732
 Thr Ile Pro Gly Met Val Gly Pro Val Ile Ala Lys Ser Leu Thr Pro
 475 480 485 490

gat aac act gtt gga gaa tgg caa acc gtg ttc tat att gct gct gct 1780
 Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala Ala
 495 500 505

att aat gtt ttt ggt gcc att ttc ttt aca cta ttc gcc aaa ggt gaa 1828
 Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly Glu
 510 515 520

gta caa aac tgg gct ctc aat gat cac cat gga cac aga cac 1870
 Val Gln Asn Trp Ala Leu Asn Asp His His Gly His Arg His
 525 530 535

tgaaggaacc aataaataat cctgcctcta ttaatgtatt tttatttatac atgtaacctc 1930

aaagtgcctt ctgtattgtg taagcattct atgtcttttt ttaattgtac ttgtattaga 1990

tttttaaggc ctataatcat gaaatatcac tagttgccag aataataaaa tgaactgtgt 2050

ttaattatga ataatatgta agctaggact tctacttttag gttcacatac ctgcctgcta 2110

gtcgggcaac atgaagtagg acagttctgt tgattttttta gggccatact aaaggggaatg 2170

agctgaaaca gacctcctga tacctttgct taattaaact agatgataat tctcaggtac 2230

tgataaacac ctgttgttgt tcaactttcct cataaaaatt gtcagctctc tctgacactt 2290

agacctcaaa ctttagcatc tctgtggagc tgccatccac tgtataattt cgcttggeaa 2350

ctggactgag gggagtgtgc ccaggcagct gccaaagcact cctccctggt cttcagggtc 2410
 agagtgccca gcgtttatca gaggcagcat ccaagcccag agccagtgtc gactcttcgg 2470
 ctgggtgcctt tctcttgagg ggctatcaat gtgtagataa agccctgagt aggcaagagc 2530
 agtgagatcc actgctatgg tcttgatata tctctaaact ttcccttccc agcacagagg 2590
 aatattggct ggcatgcaac ctgcaaaaga aaaatgcaa gcggccgggc acggtggctc 2650
 atgcctgtaa tcccagcact ttggggggct gaggtgggag aatcatgaga tcaggagttc 2710
 gagaccagcc tggccagcat ggtgaaaccc catctctact aaaaatacaa aaaattagct 2770
 gggcggtgtg acgggcgcct gtaatcccag atactcagga ggctgaggta ggagaatcac 2830
 ttgaacctgg gaggtggaag ttgcagttaa ccaagatcac gccactgcac tccagcctgg 2890
 gcgatggagc gagactccaa ctcaaaaaaa aaaaaaaaaa 2930

<210> 8

<211> 536

<212> PRT

<213> Homo sapiens

<400> 8

Met Ala Ala Gly Ala Met Thr Pro Pro Arg Pro Val Gln Pro Ala Arg
 1 5 10 15

Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg Ser Leu Leu Cys Gln Val
 20 25 30

Ala Ser Thr Pro Ala His Val Gly Val Met Arg Ser Pro Val Arg Asp
 35 40 45

Leu Ala Arg Asn Asp Gly Glu Glu Ser Thr Asp Arg Thr Pro Leu Leu
 50 55 60

Pro Gly Ala Pro Arg Ala Glu Ala Ala Pro Val Cys Cys Ser Ala Arg
 65 70 75 80

Tyr Asn Leu Ala Ile Leu Ala Phe Phe Gly Phe Phe Ile Val Tyr Ala
 85 90 95

Leu Arg Val Asn Leu Ser Val Ala Leu Val Asp Met Val Asp Ser Asn
 100 105 110

Thr Thr Leu Glu Asp Asn Arg Thr Ser Lys Ala Cys Pro Glu His Ser
 115 120 125

Ala Pro Ile Lys Val His His Asn Gln Thr Gly Lys Lys Tyr Gln Trp
 130 135 140

Asp Ala Glu Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr
 145 150 155 160

Ile Ile Thr Gln Ile Pro Gly Gly Tyr Val Ala Ser Lys Ile Gly Gly
 165 170 175

Lys Met Leu Leu Gly Phe Gly Ile Leu Gly Thr Ala Val Leu Thr Leu
 180 185 190

Phe Thr Pro Ile Ala Ala Asp Leu Gly Val Gly Pro Leu Ile Val Leu
 195 200 205

Arg Ala Leu Glu Gly Leu Gly Glu Gly Val Thr Phe Pro Ala Met His
 210 215 220

Ala Met Trp Ser Ser Trp Ala Pro Pro Leu Glu Arg Ser Lys Leu Leu
 225 230 235 240

Ser Ile Ser Tyr Ala Gly Ala Gln Leu Gly Thr Val Ile Ser Leu Pro
 245 250 255

Leu Ser Gly Ile Ile Cys Tyr Tyr Met Asn Trp Thr Tyr Val Phe Tyr
 260 265 270

Phe Phe Gly Thr Ile Gly Ile Phe Trp Phe Leu Leu Trp Ile Trp Leu
 275 280 285

Val Ser Asp Thr Pro Gln Lys His Lys Arg Ile Ser His Tyr Glu Lys
 290 295 300

Glu Tyr Ile Leu Ser Ser Leu Arg Asn Gln Leu Ser Ser Gln Lys Ser
 305 310 315 320

Val Pro Trp Val Pro Ile Leu Lys Ser Leu Pro Leu Trp Ala Ile Val
 325 330 335

Val Ala His Phe Ser Tyr Asn Trp Thr Phe Tyr Thr Leu Leu Thr Leu
 340 345 350

Leu Pro Thr Tyr Met Lys Glu Ile Leu Arg Phe Asn Val Gln Glu Asn
 355 360 365

Gly Phe Leu Ser Ser Leu Pro Tyr Leu Gly Ser Trp Leu Cys Met Ile
 370 375 380

Leu Ser Gly Gln Ala Ala Asp Asn Leu Arg Ala Lys Trp Asn Phe Ser
 385 390 395 400

Thr Leu Cys Val Arg Arg Ile Phe Ser Leu Ile Gly Met Ile Gly Pro
 405 410 415

Ala Val Phe Leu Val Ala Ala Gly Phe Ile Gly Cys Asp Tyr Ser Leu
 420 425 430

Ala Val Ala Phe Leu Thr Ile Ser Thr Thr Leu Gly Gly Phe Cys Ser
 435 440 445

Ser Gly Phe Ser Ile Asn His Leu Asp Ile Ala Pro Ser Tyr Ala Gly
 450 455 460

Ile Leu Leu Gly Ile Thr Asn Thr Phe Ala Thr Ile Pro Gly Met Val
 465 470 475 480

Gly Pro Val Ile Ala Lys Ser Leu Thr Pro Asp Asn Thr Val Gly Glu
 485 490 495

Trp Gln Thr Val Phe Tyr Ile Ala Ala Ala Ile Asn Val Phe Gly Ala
 500 505 510

Ile Phe Phe Thr Leu Phe Ala Lys Gly Glu Val Gln Asn Trp Ala Leu
 515 520 525

Asn Asp His His Gly His Arg His
 530 535

<210> 9

<211> 1485

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: human/sheep
 consensus sequence

<220>

<221> CDS

<222> (1) .. (1485)

<400> 9

atg arg tcy ccg gtt ysr gac ytr gcc csg arc gay ggc gag gag rgc 48
 Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa
 1 5 10 15

wcg gac cgc acr cck cty ctr cmg sgc gcc ccr cgg gcs gaa scc gct 96
 Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala
 20 25 30

cca gtr tgc tgc tct gct cgt tac aac yta gca wtt ttg kcc ttt ttt 144
 Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe
 35 40 45

ggg ttc ttc rtt sts tat kca tta cgg gtg aat ctg agy gtt gcr yta 192
 Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa
 50 55 60

gtg gay atg gtr gat tca aay aca act kym raa gat aat aga ack tcc 240
 Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser
 65 70 75 80

was gmg tgt sca gag cat tct gct ccc ata aaa gtt cwt cay aay caa 288
 Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln
 85 90 95

acg ggt aar aag tac crr tgg gat gca gaa act caa gga tgg att ctc 336
 Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
 100 105 110

ggw tcy ttt tty tat ggc tac atc atc aca car att cct gga gga tat 384
 Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
 115 120 125

gtt gcc agc ara akw ggg ggg aar mtg ytg cta gga tty ggg atc ytt 432
 Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa
 130 135 140

gsy acw gct rtc ytc acc ctg ttc act ccc mty gct gca gat ttm gga 480
 Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
 145 150 155 160

gty gga scm cty rtt gya ctc agr gca cta gaa ggr cta gga gag ggt 528
 Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
 165 170 175

gty aca twt cca gcc atg cat gcc atg tgg tct tcw tgg gct ccc cct 576
 Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro
 180 185 190

ctt gaa aga agc aar ctt ctk agy att tcr tat gca gga gca car ctt 624
 Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu
 195 200 205

ggg aca gta rtt tct ctt cct ctt tct gga rta att tgc tac tat atg 672
 Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met
 210 215 220

aat tgg act tat gtc ttc tay tty ttt ggy ayt rtt gga atm wty tgg 720
 Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp
 225 230 235 240

ttt mtt ttr tgg atc tgs tta gtt agt gay aca cca saa amw cac aag 768
 Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys
 245 250 255

asa aty wcy cmy tat gaa aag gar tay att ctt tca tca tta ara aat 816
 Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn
 260 265 270

cag cty tct tca cag aag tca gtg ccg tgg rta ccy atk ytr aaa tcm 864
 Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa
 275 280 285

ctg cca ctt tgg gct aty gtm gtt gca cay ttt tct tac aac tgg act 912
 Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr
 290 295 300

ttt tat act ttr ttg acm tta ttg cct act tay atg aag gar rtc cta 960
 Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu
 305 310 315 320

agg ttc aat rtt caa gag aat ggg ttt tta tct kca kts cct tat tta 1008
 Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu
 325 330 335

ggy tst tgg tta tgt atg atc ctg tck ggt caa gct gct gac aat tta 1056
 Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
 340 345 350

agg gca ara tgg aat ttt tca act ytr tgk gtt cgm aga rtt ttt agc 1104
 Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser
 355 360 365

ctt ata ggr atg att gga cct gcr rta ttc ctg gtw gcy gcw ggm tty 1152
 Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa
 370 375 380

atw ggc tgt gat tat tcy ttg gcy gtt gcw ttc cta acy ata tca aca 1200
 Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr
 385 390 395 400

acm ctg gga ggc ttt tgc tct tct gga ttt agc atc aac cat ctg gay 1248
 Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa
 405 410 415

att gct cct tcg tat gct ggt aty ctc ctg ggc atc aca aat acm ttt 1296
 Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe
 420 425 430

gcc act att ccw gga atg rtt ggg ccc rtc att gcy ara agt ctk acc 1344
 Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr
 435 440 445

cct gak aac act rtt gga gaa tgg caa acy gtk ttc try aty gct gct 1392
 Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala
 450 455 460

gct aty aat gtw ttt ggt gcc att ttc tty aca cta ttc gcc aaa ggt 1440
 Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly
 465 470 475 480

gaa gtr caa aac tgg gcy mtc art gat cac caw gga cac aga mac 1485
 Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa
 485 490 495

<210> 10

<211> 495

<212> PRT

<213> Artificial Sequence

<400> 10

Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa
 1 5 10 15

Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala
 20 25 30

Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe
 35 40 45

Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa
 50 55 60

Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser
 65 70 75 80
 Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln
 85 90 95
 Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
 100 105 110
 Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
 115 120 125
 Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa
 130 135 140
 Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
 145 150 155 160
 Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
 165 170 175
 Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro
 180 185 190
 Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu
 195 200 205
 Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met
 210 215 220
 Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp
 225 230 235 240
 Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys
 245 250 255
 Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn
 260 265 270
 Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa
 275 280 285
 Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr
 290 295 300
 Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu
 305 310 315 320

Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu
 325 330 335
 Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
 340 345 350
 Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser
 355 360 365
 Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa
 370 375 380
 Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr
 385 390 395 400
 Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa
 405 410 415
 Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe
 420 425 430
 Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr
 435 440 445
 Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala
 450 455 460
 Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly
 465 470 475 480
 Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa
 485 490 495

<210> 11

<211> 1485

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: human/sheep
 consensus sequence

<220>

<221> CDS

<222> (1) .. (1485)

<400> 11

atg ang tcn ccg gtt nnn gac ntn gcc cng anc gan ggc gag gag ngc 48
 Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa
 1 5 10 15
 ncg gac cgc acn ccn ctn ctn cng ngc gcc ccn cgg gcg gaa ncc gct 96
 Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala
 20 25 30
 cca gtn tgc tgc tct gct cgt tac aac nta gca ntt ttg ncc ttt ttt 144
 Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe
 35 40 45
 ggt ttc ttc ntt ntn tat nca tta cgn gtg aat ctg agn gtt gcg nta 192
 Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa
 50 55 60
 gtg gan atg gtn gat tca aan aca act nnn naa gat aat aga acn tcc 240
 Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser
 65 70 75 80
 nan gng tgt nca gag cat tct gct ccc ata aaa gtt cnt can aan caa 288
 Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln
 85 90 95
 acg ggt aan aag tac cnn tgg gat gca gaa act caa gga tgg att ctc 336
 Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
 100 105 110
 ggn tcn ttt ttn tat ggc tac atc atc aca can att cct gga gga tat 384
 Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
 115 120 125
 gtt gcc agc ana ann ggg ggg aan ntg ntg cta gga ttn ggg atc ntt 432
 Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa
 130 135 140
 gnn acn gct ntc ntc acc ctg ttc act ccc ntn gct gca gat ttn gga 480
 Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
 145 150 155 160
 gtn gga ncn ctn ntt gna ctc agn gca cta gaa ggn cta gga gag ggt 528
 Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
 165 170 175
 gtn aca tnt cca gcc atg cat gcc atg tgg tct tcn tgg gct ccc cct 576
 Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro
 180 185 190

ctt gaa aga agc aan ctt ctn agn att tcn tat gca gga gca can ctt 624
 Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu
 195 200 205

ggg aca gta ntt tct ctt cct ctt tct gga nta att tgc tac tat atg 672
 Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met
 210 215 220

aat tgg act tat gtc ttc tan ttn ttt ggn ant ntt gga atn ntn tgg 720
 Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp
 225 230 235 240

ttt ntt ttn tgg atc tgn tta gtt agt gan aca cca naa ann cac aag 768
 Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys
 245 250 255

ana atn ncn cnn tat gaa aag gan tan att ctt tca tca tta ana aat 816
 Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn
 260 265 270

cag ctn tct tca cag aag tca gtg ccg tgg nta ccn atn ntn aaa tcn 864
 Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa
 275 280 285

ctg cca ctt tgg gct atn gtn gtt gca can ttt tct tac aac tgg act 912
 Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr
 290 295 300

ttt tat act ttn ttg acn tta ttg cct act tan atg aag gan ntc cta 960
 Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu
 305 310 315 320

agg ttc aat ntt caa gag aat ggg ttt tta tct nca ntn cct tat tta 1008
 Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu
 325 330 335

ggn tnt tgg tta tgt atg atc ctg tcn ggt caa gct gct gac aat tta 1056
 Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
 340 345 350

agg gca ana tgg aat ttt tca act ntn tgn gtt cgn aga ntt ttt agc 1104
 Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser
 355 360 365

ctt ata ggn atg att gga cct gcg nta ttc ctg gtn gcg gcg ggn ttn 1152
 Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa
 370 375 380

atn ggc tgt gat tat tcn ttg gcn gtt gcn ttc cta acn ata tca aca 1200
 Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr
 385 390 395 400
 acn ctg gga ggc ttt tgc tct tct gga ttt agc atc aac cat ctg gan 1248
 Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa
 405 410 415
 att gct cct tcg tat gct ggt atn ctc ctg ggc atc aca aat acn ttt 1296
 Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe
 420 425 430
 gcc act att ccn gga atg ntt ggg ccc ntc att gcn ana agt ctn acc 1344
 Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr
 435 440 445
 cct gan aac act ntt gga gaa tgg caa acn gtn ttc tnn atn gct gct 1392
 Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala
 450 455 460
 gct atn aat gtn ttt ggt gcc att ttc ttn aca cta ttc gcc aaa ggt 1440
 Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly
 465 470 475 480
 gaa gtn caa aac tgg gcn ntc ant gat cac can gga cac aga nac 1485
 Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa
 485 490 495

<210> 12

<211> 495

<212> PRT

<213> Artificial Sequence

<400> 12

Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa
 1 5 10 15

Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala
 20 25 30

Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe
 35 40 45

Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa
 50 55 60

Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser

65	70	75	80
Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln	85	90	95
Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu	100	105	110
Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr	115	120	125
Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa	130	135	140
Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly	145	150	155
Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly	165	170	175
Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro	180	185	190
Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu	195	200	205
Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met	210	215	220
Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp	225	230	235
Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys	245	250	255
Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn	260	265	270
Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa	275	280	285
Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr	290	295	300
Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu	305	310	315
Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu			320

325 330 335
Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
340 345 350
Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser
355 360 365
Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa
370 375 380
Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr
385 390 395 400
Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa
405 410 415
Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe
420 425 430
Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr
435 440 445
Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala
450 455 460
Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly
465 470 475 480
Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa
485 490 495

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/16676

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/705 G01N33/50 A61K38/17 //C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL - EMBEST20 'Online! Entry HS1173506, Acc.no. AA258513, 19 March 1997 (1997-03-19). HILLIER, L. ET AL.: "zr59d01.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 667681 5' similar to TR:G507415 G507415 BRAIN SPECIFIC NA+-DEPENDENT INORGANIC PHOSPHATE COTRANSPORTER." XP002121520 the whole document</p> <p style="text-align: center;">--- -/--</p>	1,4-6

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

5 November 1999

Date of mailing of the international search report

17/11/1999

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/16676

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>HELLERQVIST C G ET AL: "ANTITUMOR EFFECTS OF GBS TOXIN: A POLYSACCHARIDE EXOTOXIN FROM GROUP B BETA-HEMOLYTIC STREPTOCOCCUS" JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY, vol. 120, no. 1/02, 1 January 1993 (1993-01-01), pages 63-70, XP000749401 ISSN: 0171-5216 the whole document</p>	1-20, 23, 25, 26, 28-40
Y	<p>GEARING, D.P. ET AL.: "Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor." EMBO JOURNAL, vol. 8, no. 12, 1989, pages 3667-76, XP002121518 abstract</p>	1-20, 23, 25, 26, 28-40
P,X	<p>FU, C. ET AL.: "Expressional cloning of CM101 receptor gene from mammalian cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION OF CANCER RESEARCH, vol. 40, March 1999 (1999-03), pages 557-Abstr.3677, XP002121519 the whole document</p>	1-15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/16676

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION SHEET PCT/ISA/210
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claims 24, 39, 40 and 44 could not be searched to completion due to insufficient characterization of the inhibitors in the description.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99 16676

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 39, and 40 in as far as it pertains to an in vivo method, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claim 22, in as far as it relates to a method for use in vivo, is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims 24, 39, 40 and 44 could not be searched to completion due to insufficient characterization of the inhibitors in the description.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/12, C07K 14/705, G01N 33/50, A61K 38/17 // C07K 16/28	A1	(11) International Publication Number: WO 00/05375 (43) International Publication Date: 3 February 2000 (03.02.00)
(21) International Application Number: PCT/US99/16676 (22) International Filing Date: 22 July 1999 (22.07.99) (30) Priority Data: 60/093,843 22 July 1998 (22.07.98) US (71) Applicant (for all designated States except US): VAN- DERBILT UNIVERSITY [US/US]; 305 Kirkland Hall, Nashville, TN 37240 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HELLERQVIST, Carl, G. [US/US]; 9014 Carondelet Place, Brentwood, TN 37027 (US). FU, Changlin [CN/US]; 5568 Saddlewood Lane, Brentwood, TN 37027 (US). (74) Agents: ASHTON, Nina, M.; Cooley Godward LLP, 3000 El Camino Real, Five Palo Alto Square, Palo Alto, CA 94306-2155 (US) et al.		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: GBS TOXIN RECEPTOR (57) Abstract A novel GBS toxin receptor, and methods for its preparation and use are provided. GBS toxin receptor polynucleotides and polypeptides are provided as well as detection, screening, and therapeutic methods and pharmaceutical compositions involving such polynucleotides and polypeptides.		

*(Referred to in PCT Gazette No. 46/2000, Section II)

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Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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GBS TOXIN RECEPTOR

INTRODUCTIONTechnical Field

5 This invention provides compositions and methods relating to GBS toxin receptor polynucleotides and polypeptides. The invention relates to a receptor for a polysaccharide isolated from a bacterial source.

Background

 Group B β -hemolytic Streptococci (GBS) are ubiquitous microorganisms.
10 GBS is not known to cause any harmful infections in humans except for very young babies. GBS pneumonia, also called "early-onset disease", is associated with high morbidity and mortality in newborn infants.

 In a series of studies conducted by Dr. Carl G. Hellerqvist and his associates at the Vanderbilt University School of Medicine, Nashville, Tennessee, a polysaccharide
15 GBS toxin was identified. This toxin was determined to be a major factor in the complications of GBS pneumonia, and was found to be useful as a therapeutic agent in combating tumors through inhibition of vascularization (U.S. Patent No. 5,010,062).

 In addition, as described in U.S. Patent No. 5,858,991 and WO98/32453, GBS toxin facilitates wound healing in patients by minimizing scarring and accelerating
20 healing, and reduces wound-related tumor progression.

 WO98/32452 and WO98/32448 describe the use of GBS toxin as a therapeutic agent for treating patients with chronic inflammatory diseases, such as rheumatoid arthritis and psoriasis, and for enhancing repair of neural injury.

 Prior to this invention, receptors for GBS toxin had not been identified. The
25 inventors, believing receptors of GBS toxin to reside on cells in the developing vasculature of tissues undergoing angiogenesis in the conditions described above, embarked upon a series of experiments resulting in the present invention.

SUMMARY OF THE INVENTION

For the first time, novel receptors for group B β -hemolytic Streptococcus GBS toxin (GBS toxin receptor) have been identified. One aspect of the invention provides a polypeptide comprising a GBS toxin receptor or polypeptide fragment thereof.

- 5 Preferred embodiments include mammalian GBS toxin receptors. Also provided is an antibody that recognizes GBS toxin receptor or a fragment thereof. The polypeptide of the invention can be used, *inter alia*, for the screening of compounds that can be used to treat or prevent conditions arising from pathologic or hypoxia-driven angiogenesis or neovascularization, such as, for example, cancerous tumors, chronic
10 inflammatory disease, scarring during wound healing, keloids, neural injury, and reperfusion injury.

- Another aspect of the invention provides a polynucleotide encoding a GBS toxin receptor or a fragment thereof and a polynucleotide hybridizable to such polynucleotide. Preferred polynucleotides are at least 10 bases in length and comprise
15 a nucleic acid sequence encoding, or are complementary to a nucleic acid sequence encoding, a mammalian GBS toxin receptor or a polypeptide fragment thereof.

- A third aspect of the invention is a complex comprising a GBS toxin bound to a mammalian toxin receptor or fragment thereof. Also provided is a method of forming such complex. The method comprises contacting a GBS toxin with a
20 polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide, and allowing the complex to form.

- Yet another aspect of the invention is a method for purifying a compound that binds a GBS toxin receptor. The method comprises providing a polypeptide
25 comprising a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, contacting the polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide, and separating the bound compound from the remainder of the sample.

- Another aspect of the invention is a method of determining the presence or
30 absence of GBS toxin in a sample. The method comprises contacting the sample with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, under conditions that allow specific binding of GBS toxin to the GBS toxin receptor, and determining whether specific binding of GBS toxin has

occurred. Presence of GBS toxin in a sample obtained from a neonate is indicative of early onset disease.

A sixth aspect of the invention is a method for detecting pathologic vasculature in a mammalian tissue. The method comprises detecting the presence of a GBS toxin receptor. The method can be used for detecting or monitoring a variety of medical conditions associated with angiogenesis or neovascularization, such as, for example, detecting metastasis of a cancerous tumor, or monitoring the margin of a tumor in a mammal undergoing a therapy for cancer.

Another aspect of the invention provides methods for the identification of drug candidates for the treatment of medical conditions characterized by pathologic and/or hypoxia-driven angiogenesis or neovascularization. One embodiment is a method for identifying a compound that specifically binds a mammalian GBS toxin receptor. The method comprises combining a test compound with a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that allow specific binding to occur, and detecting a complex formed between the test compound and the polypeptide. Another embodiment is a method for determining cytotoxicity of a test chimeric compound. The method comprises exposing a cell expressing a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, to a test chimeric compound comprising a cytotoxic agent coupled to GBS toxin, and detecting signs of toxicity. Yet another embodiment is a method for identifying an inhibitor of a GBS toxin receptor by incubating test cells that express GBS toxin receptor, or a fragment thereof, in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, and comparing the proliferation or migration of the test cells incubated in the presence and absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor. An inhibitor of endothelial cell proliferation or migration can be identified by the above method, wherein less proliferation or migration of test cells in the presence of the test compound is indicative of the test compound being an inhibitor of endothelial cell proliferation or migration. A therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic angiogenesis or neovascularization can also be identified by the above method, wherein less proliferation or migration of test cells in the presence of the test compound is indicative of the test compound being a candidate

therapeutic compound for the treatment or prevention of the medical condition.

The invention also provides a method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor. The method comprises simulating and selecting the most probable conformations of a mammalian GBS toxin receptor, designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the polypeptide, chemically synthesizing the analog, and evaluating the bioactivity of the analog. Also provided is a method for identifying a compound which binds to a mammalian GBS toxin receptor. The method comprises simulating and selecting the most probable conformations of a mammalian GBS toxin receptor, deducing the most probable binding domains of the polypeptide, designing a compound that would form the energetically most probable complexes with the polypeptide, chemically synthesizing the compound, and evaluating the bioactivity of the compound.

Another aspect of the invention is a method for the prevention or treatment of neonatal onset disease in a human neonate by administering an inhibitor of binding of GBS toxin to a human GBS toxin receptor.

Yet another aspect of the invention is a method for inhibiting pathologic or hypoxia-driven endothelial cell proliferation or migration in a mammalian tissue. The method comprises specifically binding a molecule to a GBS toxin receptor present on the surface of at least one cell in the tissue, the molecule being selected from the group consisting of a compound that can evoke an inflammatory response when bound to a GBS toxin receptor in a mammal, a chimeric compound comprising a cytotoxic compound coupled to a compound that specifically binds the GBS toxin receptor, an inhibitor of GBS toxin receptor phosphorylation, and an inhibitor of GBS toxin receptor activity.

The invention also provides a GBS toxin receptor or fragment thereof, an inhibitor of a GBS toxin receptor, or an inhibitor of binding of a GBS toxin to a GBS toxin receptor, for use in a method of treatment of the human or animal body or for the manufacture of a medicament for the treatment of a medical condition characterized by pathologic or hypoxia-driven angiogenesis or neovascularization. Also provided is a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor for use in a method of treatment of the human or animal body.

Also provided are pharmaceutical compositions comprising an inhibitor of a

GBS toxin receptor and/or a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor, and a pharmaceutically acceptable carrier.

- The invention also provides kits comprising a GBS toxin receptor or fragment and/or reagents for detecting the presence of a GBS toxin receptor or polypeptide fragment thereof or the presence of a polynucleotide encoding same.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a process of rational drug design.

FIGS. 2A and 2B depict the results of immunohistochemical analysis of GBS toxin receptor expression in cancerous and normal human ovary tissue, respectively, using antibody Pab55 as described in Example 4.

FIGS. 3A and 3B depict the results of immunohistochemical analysis of GBS toxin receptor expression in cancerous and normal human ovary tissue, respectively, using antibody Pab57 as described in Example 4.

FIGS. 4A-4C depict the targeted delivery of a chimeric compound to GBS toxin receptor expressed in a cancerous tissue as described in Example 6.

DESCRIPTION OF SPECIFIC EMBODIMENTS

DEFINITIONS

Generally, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, and microbial culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification steps supplied by manufacturers are typically performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references (See generally, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring

Harbor, N.Y.) which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical chemistry, organic synthetic chemistry, and pharmaceutical formulation described below are those well known and commonly employed in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical formulation and delivery, and treatment of patients. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

By "GBS toxin receptor" is meant a proteinaceous molecule capable of binding a toxin from Group B β -hemolytic *Streptococcus* bacteria (GBS toxin), such as, for example, CM101. A GBS toxin receptor is usually found in nature on the surface of a cell. Recombinant membrane bound and soluble GBS toxin receptors can be produced by laboratory techniques known in the art and described herein.

The term "isolated polynucleotide" referred to herein means a polynucleotide that has been subjected to manipulation, such that the isolated polynucleotide is no longer associated with the chromosome or cell that the polynucleotide is normally associated with in nature in the same manner as it is normally associated in nature. An example of an "isolated polynucleotide" is a polynucleotide of genomic, recombinant, or synthetic origin or some combination thereof.

The term "isolated protein" referred to herein means a protein that is no longer associated with the cell that the protein is normally associated with in nature in the same manner as it is normally associated in nature, such as (1) a protein free of at least some other proteins from the same source, (2) a protein expressed by a cell from a different species, (3) a protein that does not occur in nature, and (4) a protein produced from cDNA, recombinant RNA, or synthetic origin or some combination thereof.

The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus.

The term "naturally occurring" means found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) found in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "operably linked" refers to a juxtaposition wherein the components

so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

5 The term "control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control
10 sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is necessary for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

15 The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. The term includes single- and double-stranded forms of DNA.

 The term "oligonucleotide" referred to herein includes naturally occurring, and
20 modified nucleotides linked together by naturally occurring and non-naturally occurring oligonucleotide linkages. An oligonucleotide is usually a polynucleotide 200 bases or fewer in length. Preferably oligonucleotides are minimally 10 to 60 bases in length and most preferably
15-35 bases in minimal length. Oligonucleotides are usually single-stranded, e.g. for
25 probes; although oligonucleotides may be double-stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides. The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified
nucleotides" referred to herein includes nucleotides with modified or substituted sugar
30 groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. An oligonucleotide can include a label for detection, if desired.

By "complementary" or "complement" is meant that wherever adenine appears in a first nucleic acid sequence, thymine or uracil is found in the "complementary" sequence and vice versa, and wherever guanine appears in a first nucleic acid sequence, cytosine is found in the "complementary" sequence and vice versa.

The term "sequence identity" describes the proportion of base matches between two nucleic acid sequences or the proportion of amino acid matches between two amino acid sequences, i.e. the degree of identity between two sequences. When sequence identity is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of exact matches over the length of sequence from a GBS toxin receptor sequence that is compared to some other sequence. Various computer alignment programs can be used to determine sequence identity. In its simplest form, % identity is calculated by dividing the number of exact matches between two nucleic acid sequences or between two amino acid sequences by the total number of nucleotides or amino acids in the reference sequence. For example, if there are 300 matches between sequences 400 amino acids in length, the sequences have 75% identity. Uracil and thymine are considered identical when comparing a ribonucleic acid sequence with a deoxyribonucleic acid sequence.

As applied to polynucleotides, the term "substantial identity" means that two nucleic acid sequences when optimally aligned, such as by the program BLAST (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990)), share at least about 85%, preferably at least about 90% sequence identity and most preferably 95% or greater sequence identity. When using computer alignment programs, gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less are usually used; 6 bases or less are preferred; 2 bases or less are most preferred. When using oligonucleotides as probes or in treatments, the sequence identity between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and most preferably not less than 19 matches out of 20 possible base pair matches (95%).

Preferably, bases which are not identical nevertheless are part of a degenerate codon that encodes the same amino acid at that amino acid position. Alternatively, bases which are not identical preferably are part of a degenerate codon that encodes a conservative amino acid substitution for that amino acid position.

5 As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned by the BLAST computer program, share at least about 80 percent sequence identity, preferably at least about 86 percent sequence identity, more preferably at least about 95 percent sequence identity, even more preferably at least about 99 percent sequence identity up to having one amino acid
10 difference, and most preferably share 100% identity. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side
15 chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino
20 acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

25 The term "hybridizable under high stringency conditions" referred to herein means capable of specific binding under conditions whereby only nucleic acid sequences having a substantial identity of greater than 95% with respect to each other will hybridize. These conditions are known in the art and discussed herein.

The term "degenerate codon" means any of the nucleotide codon triplets
30 encoding a desired amino acid according to the genetic code. Codons can be selected based upon known preferred codon usage in a host organism such as *E. coli*.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-

occurring sequence deduced, for example, from a full-length DNA sequence.

Fragments typically are at least 3 amino acids long, preferably are 5-10 amino acids long, more preferably are 10-50 amino acids long, even more preferably are more than 50 amino acids long and comprise at least one extracellular domain of a GBS toxin receptor. Most preferred are fragments that comprise the entire extracellular domains of a GBS toxin receptor, and preferably also comprise portions of transmembrane and intracellular domains sufficient to maintain the polypeptide fragment in a functional stereochemical conformation on the surface of a cell, lipid membrane, liposome, micelle, or other lipophilic structure.

10 The term "immunologically reactive" means having antigenic properties or being capable of being specifically bound by an antibody that can specifically bind GBS toxin receptor. A substance has antigenic properties if it can generate monoclonal or polyclonal antibodies when administered to an animal under conditions known in the art to facilitate the production of antibodies that will recognize and bind
15 a particular antigen.

A "heterologous polypeptide" is a polypeptide different from polypeptides normally produced by a particular cell. For example, a GBS toxin receptor polypeptide or fragment thereof that is produced recombinantly in a cell that does not normally produce such GBS toxin receptor polypeptide or fragment thereof, is a
20 heterologous polypeptide. A second polypeptide joined to a GBS toxin receptor polypeptide or fragment thereof is also a heterologous polypeptide if it is not joined to a GBS toxin receptor polypeptide in nature.

As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to
25 a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes (e.g., ^3H , ^{14}C ,
30 ^{35}S , ^{125}I , ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some

embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The term "compound" as used herein preferably refers to a peptidic, peptidomimetic, organic, or other chemical molecule and also refers to a nucleic acid molecule or chemical derivative thereof. The compound can interact with, or be, the polynucleotides or polypeptides of the invention.

The singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

The SEQ ID NOs of the nucleic acid and amino acid sequences described herein are summarized below in Table 1.

Table 1
Nucleic Acid and Amino Acid Sequences

SEQ ID NO:	Type of Sequence	Description
SEQ ID NO: 1	nucleic acid	Partial human GBS toxin receptor (HP55)
SEQ ID NO: 2	amino acid	Partial human GBS toxin receptor (HP55)
SEQ ID NO: 3	nucleic acid	Sheep GBS toxin receptor (SP55)
SEQ ID NO: 4	amino acid	Sheep GBS toxin receptor (SP55)
SEQ ID NO: 5	nucleic acid	Primer
SEQ ID NO: 6	nucleic acid	Primer
SEQ ID NO: 7	nucleic acid	Full-length human GBS toxin receptor (HP59)
SEQ ID NO: 8	amino acid	Full-length human GBS toxin receptor (HP59)
SEQ ID NO: 9	nucleic acid	Human/Sheep consensus GBS toxin receptor coding region (with base codes a, c, g, t, m, r, w, s, y, k)
SEQ ID NO: 10	amino acid	Human/Sheep consensus GBS toxin receptor coding region (translation of SEQ ID No: 9)
SEQ ID NO: 11	nucleic acid	Human/Sheep consensus GBS toxin receptor coding region (with base codes a, c, g, t, n)
SEQ ID NO: 12	amino acid	Human/sheep consensus GBS toxin receptor coding region (translation of SEQ ID NO: 11)

The headings provided herein describe the general topic discussed and are not intended to be exclusive of information discussed in other sections. Frequently, information, methods, compositions, and other aspects may be applicable to more than one embodiment of the invention and can be so combined.

INTRODUCTION

GBS toxin binds to tissues undergoing pathologic, hypoxia-driven, and

embryologic angiogenesis or neovascularization. The inventors have identified at least two mammalian GBS toxin receptors, which are described herein. Examples 1 and 2 describe the cloning and characterization of some GBS toxin receptors. The inventors have classified GBS toxin receptor as an integral protein with seven
5 transmembrane domains. The predicted segments are shown in Table 7. The protein has several putative sites for phosphorylation by cAMP-dependent kinase, protein kinase C (PKC), and casein kinase II (CK2). Typically, such integral proteins, upon binding of a molecule (e.g., a ligand or an extracellular messenger), undergo a conformational change which facilitates phosphorylation at phosphorylation sites such
10 as those discussed above. The phosphorylation of the protein at these sites may trigger a signal transduction cascade, which often results in proliferation or other nuclear responses of the cells which have been exposed to the binding molecule. Angiogenesis or neovascularization involves proliferation and migration of endothelial cells. As discussed in greater detail in Examples 4 and 5, GBS toxin
15 receptor expression is correlated with medical conditions involving pathologic, hypoxia-driven, and embryogenic angiogenesis or neovascularization. GBS toxin receptor polypeptides can be used for a variety of purposes, including screening for compounds that can inhibit endothelial cell proliferation and/or migration mediated by GBS toxin receptor and screening for cytotoxic chimeric compounds that can bind to
20 and destroy cells expressing GBS toxin receptor. GBS toxin receptor polynucleotides can be used for a variety of purposes, including the design of antisense polynucleotides that can block translation of messenger RNA encoding GBS toxin receptor.

25 POLYNUCLEOTIDES

One aspect of the invention provides for isolated polynucleotides at least ten bases in length encoding or complementary to a nucleic acid sequence encoding a GBS toxin receptor or a fragment derived therefrom. Preferably, the GBS toxin receptor is a mammalian GBS toxin receptor, more preferably an ovine, bovine or
30 feline GBS toxin receptor, and most preferably a human GBS toxin receptor. The isolated polynucleotides can be naturally occurring or non-naturally occurring. The isolated polynucleotides can comprise a DNA sequence or an RNA sequence in which every T is replaced with U. For purposes of determining percentage identity, T is considered equivalent to U. Preferably, the polynucleotides include alleles of an

ovine, bovine, feline or human GBS toxin receptor, and can include alleles of GBS toxin receptor of other mammals. These polynucleotides can be isolated using polynucleotides derived from SEQ ID NOs: 1, 3, 7, 9 and 11, as described further below.

- 5 Polynucleotides, oligonucleotides and fragments of the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. The polynucleotides can be hybridizable under high stringency conditions to a nucleic acid molecule having a nucleic acid sequence comprising at least 20 contiguous
- 10 polynucleotides, preferably at least 30 contiguous nucleotides of SEQ ID NO: 1 or SEQ ID NO: 3, and even more preferably to the nucleic acid sequence of SEQ ID NO: 1, 3, 7, 9 or 11 or the complement of SEQ ID NO: 1, 3, 7, 9 or 11. Such polynucleotides can be used for performing selective, high stringency hybridization and are particularly useful for performing amplification of nucleic acid by polymerase
- 15 chain reaction (PCR) to determine the presence or absence of GBS toxin receptor in a sample, for isolating a naturally occurring nucleic acid encoding a GBS toxin receptor (see Example 3), as antisense molecules for blocking translation of GBS toxin receptor mRNA. Particularly preferred are polynucleotides hybridizable under high stringency conditions to a nucleic acid molecule having a nucleic acid sequence
- 20 comprising the nucleic acid sequence of nucleotides 266 to 1870 of SEQ ID NO: 7 (the putative full length coding region of a human GBS toxin receptor, excluding the start codon), nucleotides 266 to 1870 of SEQ ID NO: 7 (the putative full length coding region of a human GBS toxin receptor, including the start codon), nucleotides 61 to 1542 of SEQ ID NO: 1 (the partial coding region of a human GBS toxin receptor,
- 25 excluding the start codon), nucleotides 58 to 1542 of SEQ ID NO: 1 (the partial coding region of a human GBS toxin receptor, including the start codon), nucleotides 87 to 1568 of SEQ ID NO: 3 (the coding region of a sheep GBS toxin receptor, excluding the start codon), nucleotides 84 to 1568 of SEQ ID NO: 3 (the coding region of a sheep GBS toxin receptor, including the start codon), or a complementary nucleic
- 30 acid sequence thereof.

The polynucleotides can have an identity to the nucleic acid sequence of a corresponding region of SEQ ID NO: 1, 3 or 7 or the complement of a corresponding region of SEQ ID NO: 1, 3 or 7 in the range of about 85% to 100%, preferably greater than about 87% identity, more preferably greater than about 95% identity, and most

preferably about 99% to 100% identity. Particularly preferred are polynucleotides comprising the nucleic acid sequence of nucleotides 266 to 1870 of SEQ ID NO: 7, or nucleotides 87 to 1568 of SEQ ID NO: 3, SEQ ID NO: 9, SEQ ID NO: 11, or a complementary nucleic acid sequence thereof.

5 Preferably, the polynucleotides comprise a nucleic acid sequence encoding, or complementary to a nucleic acid sequence encoding, a polypeptide having an identity to the amino acid sequence of a fragment of a GBS toxin receptor in the range of about 85% to 100%, more preferably greater than 86% identity, even more preferably greater than 95% identity, and most preferably 99% to 100% identity. Preferably, the
10 fragment binds GBS toxin. Preferred fragments comprise all or a portion of residues 1 to 495 of SEQ ID NO: 2 or all or a portion of residues 1 to 536 of SEQ ID NO: 8. Particularly preferred are polynucleotides comprising a nucleic acid sequence encoding a polypeptide having 100% identity to the amino acid sequence of residues 1 to 495 of SEQ ID NO: 4, residues 1 to 495 of SEQ ID NO: 2, or residues 1 to 536 of
15 SEQ ID NO: 8.

 Polynucleotides encoding naturally occurring GBS toxin receptor can be isolated from various tissue sources and cell cultures from different species that produce such a receptor by the methods described herein, such as, for example, cells from tumor endothelium, synovial tissue in rheumatoid arthritis, or hypoxic tissue
20 deprived of or restricted from blood flow, such as in reperfusion injury or wounded tissue. Such polynucleotides can be isolated by hybridization using probes or by polymerase chain reaction using oligonucleotides, as well as by implementing other molecular biology techniques known in the art. Such probes and oligonucleotides typically comprise various regions of the sequence of SEQ ID NO: 1, 3, 7, 9 or 11,
25 preferably of SEQ ID NO: 1, 3, or 7, or encode various regions of the sequence of SEQ ID NO: 2, 4, 8, 10 or 12, preferably of SEQ NO: 2, 4 or 8.

 Polynucleotides useful for cloning genes encoding GBS toxin receptors of various organisms can be determined by comparing the amino acid sequences of homologous proteins. (see Table 4). For example, conserved regions can be targeted
30 for the synthesis of oligonucleotides or degenerate oligonucleotides to be used as probes for hybridization or nucleic acid amplification, techniques discussed further below and in Example 3. Stringency can be varied to achieve selective hybridization conditions whereby nucleic acid sequences having less than 95% identity with respect to each other will hybridize. These conditions are known in the art and discussed

herein and examples are provided. Generally, the nucleic acid sequence identity between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least about 85%, and more typically with preferably increasing identities of at least about 90%, 95%, 99%, and 100%.

- 5 Polynucleotides can be used as probes under high stringency wash conditions and with corresponding hybridization conditions, as known in the art. Small polynucleotides, for example, polynucleotides 200 bases or fewer in length, are often referred to in the art as oligonucleotides. Techniques for using polynucleotides as probes to detect the same or related nucleic acid sequences is well known in the art.
- 10 See, for example, Sambrook et al, especially Chapter 11, the text of which is herein incorporated by reference. Usually, probes can be made from polynucleotides that are 10 to 200 bases in length. Preferably probes are made from polynucleotides 10 to 60 nucleotides in length and most preferably 12 to 40 bases in length. Specific probes can be designed based on results obtained using nucleic acid homology computer
- 15 programs such as FASTA, which uses the method of Pearson and Lipman (*Proc. Natl. Acad. Sci. USA* 85:2444-2448 (1988)) and shows the degree of identity between compared sequences. The size of the probe is dependent upon the region of the gene to which it will be hybridized. The size of the probe increases as the degree of homology to undesirable nucleic acid sequences increases. A probe 10-50 nucleotides
- 20 in length can be used, preferably more than 50 nucleotides, even more preferably more than 100 nucleotides, and most preferably a probe made from the entire coding region of a GBS toxin receptor will be used. To decrease the number of false positives, preferably two probes are used to identify clones that bind to both probes under hybridization and wash conditions. Oligonucleotides can be synthesized on an
- 25 Applied BioSystems oligonucleotide synthesizer according to specifications provided by the manufacturer.

- Typically, hybridization and washing conditions are performed at according to conventional hybridization procedures. Typical hybridization conditions for screening plaque lifts (Benton and Davis (1978) *Science* 196: 180) can be: 50%
- 30 formamide, 5 x SSC (sodium chloride, sodium citrate) or SSPE (sodium chloride, sodium phosphate, EDTA), 1-5 x Denhardt's solution, 0.1-1% SDS, 100-200 μ g sheared heterologous DNA or tRNA, 0-10% dextran sulfate, 1×10^5 to 1×10^7 cpm/ml of denatured probe with a specific activity of about 1×10^8 cpm/ μ g, and

incubation at 42°C for about 6-36 hours. Prehybridization conditions are essentially identical except that probe is not included and incubation time is typically reduced. Washing conditions are typically 1-3 x SSC, 0.1-1% SDS, 42-70°C with change of wash solution at about 5-30 minutes. Cognate bacterial sequences, including allelic sequences, can be obtained in this manner. For high stringency hybridization conditions, various parameters can be altered to increase the stringency of hybridization, such as by increasing the temperature of incubation with the labeled probe. Preferably, for greater flexibility in experimental design, the probe can be hybridized at a lower temperature, such as, for example, room temperature and the stringency can then be modified by altering the salt concentration and temperature of the wash solutions. For high stringency a wash temperature of greater than or equal to 42°C can be used, such as, for example, 68°C, in a wash buffer having a salt concentration less than 3X SSC, such as, for example, 0.1X SSC. In some cases, TMACL can also be used, particularly for polynucleotides rich in G-C base pairs in order to decrease non-specific binding. A lower stringency wash can be used to hybridize polynucleotides with lower identities or polynucleotides that are less than 60 base pairs in length. For a low stringency wash, temperatures of less than or equal to 42° can be used in a wash buffer having a salt concentration of greater than or equal to 2X SSC.

The invention includes methods for amplification of target nucleic acids, such as the polymerase chain reaction ("PCR") technique. The PCR technique can be applied to identify related sequences in the genomes of various organisms and to detect nucleotide sequences in suspected samples, using oligonucleotide primers spaced apart from each other and based on the genetic sequence set forth herein. The primers are complementary to opposite strands of a double-stranded DNA molecule and are typically separated by from about 50 to 450 nucleotides or more (usually not more than 2000 nucleotides). This method entails preparing the specific oligonucleotide primers followed by repeated cycles of target DNA denaturation, primer binding, and extension with a DNA polymerase to obtain DNA fragments of the expected length based on the primer spacing. Extension products generated from one primer serve as additional target sequences for the other primer. The degree of amplification of a target sequence is controlled by the number of cycles that are performed and is theoretically calculated by the simple formula 2^n where n is the number of cycles. Given that the average efficiency per cycle ranges from about 65%

to 85%, 25 cycles produce from 0.3 to 4.8 million copies of the target sequence. The PCR method is described in a number of publications, including Saiki et al., *Science* (1985) 230:1350-1354; Saiki et al., *Nature* (1986) 324:163-166; and Scharf et al., *Science* (1986) 233:1076-1078. Also see U.S. Patent Nos. 4,683,194; 4,683,195; and 4,683,202, the text of each patent is herein incorporated by reference. Additional methods for PCR amplification are described in: PCR Technology: Principles and Applications for DNA Amplification ed. HA Erlich, Freeman Press, New York, NY (1992); *PCR Protocols: A Guide to Methods and Applications*, eds. Innis, Gelfand, Snisky, and White, Academic Press, San Diego, CA (1990); Mattila et al. (1991) *Nucleic Acids Res.* 19: 4967; Eckert, K.A. and Kunkel, T.A. (1991) *PCR Methods and Applications I*: 17, and; *PCR*, eds. McPherson, Quirkles, and Taylor, IRL Press, Oxford, all of which are incorporated herein by reference.

In yet another embodiment, an antisense polynucleotide can be administered to a mammal to treat or prevent a medical condition involving pathologic and/or hypoxia-driven angiogenesis. The antisense oligonucleotides of the invention can be synthesized by any of the known chemical oligonucleotide synthesis methods. Such methods are generally described, for example, in Winnacker, *From Genes to Clones: Introduction to Gene Technology*. VCH Verlagsgesellschaft mbH (H., Ibelgauf trans. 1987). Any of the known methods of oligonucleotide synthesis can be utilized in preparing the instant antisense oligonucleotides. The antisense oligonucleotides are most advantageously prepared by utilizing any of the commercially available, automated nucleic acid synthesizers. The device utilized to prepare the oligonucleotides described herein, the Applied Biosystems 380B DNA Synthesizer, utilizes -cyanoethyl phosphoramidite chemistry. Antisense oligonucleotides hybridizable with any portion of the mRNA transcript can be prepared by the oligonucleotide synthesis methods known to those skilled in the art. While any length oligonucleotide can be utilized in the practice of the invention, sequences shorter than 12 bases may be less specific in hybridizing to the target GBS toxin receptor mRNA, and may be more easily destroyed by enzymatic digestion. Hence, oligonucleotides having 12 or more nucleotides are preferred. Sequences longer than 18 to 21 nucleotides may be somewhat less effective in inhibiting GBS toxin receptor translation because of decreased uptake by the target cell. Thus, oligomers of 12-21 nucleotides are most preferred in the practice of the present invention, particularly oligomers of 12-18 nucleotides. Oligonucleotides complementary to and hybridizable

with any portion of the GBS toxin receptor mRNA transcript are, in principle, effective for inhibiting translation of the transcript, and capable of inducing the effects herein described. Translation is most effectively inhibited by blocking the mRNA at a site at or near the initiation codon. Thus, oligonucleotides complementary to the 5' region of the GBS toxin receptor mRNA transcript are preferred. Secondary or tertiary structure which might interfere with hybridization is minimal in this region. Moreover, sequences that are too distant in the 3' direction from the initiation site can be less effective in hybridizing the mRNA transcripts because of a "read-through" phenomenon whereby the ribosome is postulated to unravel the antisense/sense duplex to permit translation of the message. (see, e.g. Shakin, J. Biochemistry 261, 16018 (1986)). The antisense oligonucleotide is preferably directed to a site at or near the ATG initiation codon for protein synthesis. Oligonucleotides complementary to a portion of the GBS toxin receptor mRNA including the initiation codon are preferred. While antisense oligomers complementary to the 5' region of the GBS toxin receptor transcript are preferred, particularly the region including the initiation codon, it should be appreciated that useful antisense oligomers are not limited to those complementary to the sequences found in the translated portion of the mRNA transcript, but also includes oligomers complementary to nucleotide sequences contained in, or extending into, the 5' and 3' untranslated regions. Antisense nucleotides or antisense expression constructs can find use to treat or prevent diseases associated with pathologic or hypoxia-driven angiogenesis and neovascularization, as inappropriate expression of GBS toxin receptor results in hyperproliferation of endothelial cells.

In one embodiment, the polynucleotides of the invention can exist in linear form. In another embodiment, the polynucleotides can exist in circular form as part of a plasmid.

In yet another embodiment, a probe or PCR primer comprises a group of polynucleotide species containing different degenerate codons at various positions, which polynucleotides encode, or are complementary to sequences encoding, a GBS toxin receptor in whole or in part. Such polynucleotides can be useful for isolating nucleic acid sequences encoding polypeptides having at least about 85% identity to the amino acid sequence of sheep or human GBS toxin receptor, such as, for example, GBS toxin receptors of other organisms. Typically, such polynucleotides are synthesized chemically as described above by programming a synthesizer to incorporate a particular combination of nucleic acid residues at a certain position.

Typical designations are shown in Table 2.

Table 2
Base Codes

<u>Symbol</u>	<u>Meaning</u>
A	A: adenine
C	C; cytosine
G	G; guanine
T	T; thymine
U	U; uracil
M	A or C
R	A or G
W	A or T/U
S	C or G
Y	C or T/U
K	G or T/U
V	A or C or G; not T/U
H	A or C or T/U; not G
D	A or G or T/U; not C
B	C or G or T/U; not A
N	A or C or G or T/U

POLYPEPTIDES

Another aspect of the invention provides polypeptides comprising (1) the full length GBS toxin receptor protein or a naturally occurring allelic variant thereof, (2) fragments of at least 3 amino acids of the amino acid sequence of SEQ ID NO: 2, 4, 8, 10 or 12, and (3) a GBS toxin receptor protein, polypeptide, or polypeptide fragment having an amino acid identity in the range of about 80% to 100% to the amino acid sequence of a corresponding region of SEQ ID NO: 2, 4 or 8. Preferred fragments of the amino acid sequence of SEQ ID NO: 2, 4, 8, 10 or 12, are at least 5, 6, 7, 8 or 9 amino acids in length and are immunologically reactive, i.e., immunogenic. More preferred are fragments at least 25 amino acids in length and fragments comprising the amino acid sequence of residues 181 to 419 of SEQ ID NO: 2 or residues 1 to 240 of SEQ ID NO: 4. Most preferred are fragments that can bind GBS toxin. Preferably, the GBS toxin receptor protein, polypeptide, or polypeptide fragment has an amino acid identity to the amino acid sequence of a corresponding region of SEQ ID NO: 2, 4 or 8 of at least about 86%, more preferably at least about 95% identity, even more preferably at least about 99% identity up to having one amino acid difference, and most preferably 100% identity. Preferred polypeptides have at least about 89% identity, more preferably at least about 95% identity, even more preferably at least about 99% identity up to having one amino acid difference, and most preferably 100% identity to the amino acid sequence of residues 181 to 419 of SEQ ID NO: 2, residues 1 to 495 of SEQ ID NO: 4. Preferably, a full length GBS toxin receptor protein comprises the amino acid sequence of residues 1 to 495 of SEQ ID NO: 2, residues 1 to 495 of SEQ ID NO: 4, or residues 1 to 536 of SEQ ID NO: 8, or an allelic variant thereof. The polypeptides of the invention can include amino acids in addition to the GBS toxin receptor protein, polypeptide, or polypeptide fragment. Such polypeptides typically comprise a heterologous polypeptide joined to a second polypeptide derived, as described above, from a GBS toxin receptor. Preferably the additional amino acids are covalently linked to the amino-terminal or carboxy-terminal terminus of the GBS toxin receptor protein, polypeptide, or polypeptide fragment.

Fragments or analogs of GBS toxin receptor can be prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. For example, such functional domains include domains conferring the property of induction of an inflammatory response upon binding of GBS toxin to the GBS toxin receptor. GBS toxin mediates

the binding and opsonization by C3 of endothelial cells that express the GBS toxin receptor. Such domains can comprise the binding site for GBS toxin, in whole or in part, or domains otherwise essential for GBS toxin receptor structure and/or function. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known (Bowie et al. (1991) *Science* 253: 164). Computerized prediction methods, such as, for example, a hydropathy profile as provided by the "Soap" program in PC/GENE can be employed to identify putative structural and functional domains. Using the method of Klein, Kanehisa and DeLise, Biochim Biophys Acta (1985) 815:468-476, the inventors have classified a sheep GBS toxin receptor, SP55, as an integral protein with seven transmembrane segments predicted. Such a protein is also known colloquially in the art as a "7-spanner". The predicted segments are set forth below in Table 3.

15

Table 3

Predicted Transmembrane Domains of SP55

No.	Inner Boundaries		Outer Boundaries		Segment Sequence	P:I odds*
	From	To	From	To		
1	232	248	226	252	FFGIVGIIWFILWICLV (232-248 of SEQ ID No. 4)	2.589323E-05
2	369	385	365	389	LIGMIGPAIFLVAAGFI (369-385 of SEQ ID No. 4)	1.007311E-03
3	458	474	456	479	TVFCIAAAINVFGAIFF (458-474 of SEQ ID No. 4)	2.482542E-03
4	137	153	135	157	LLLGFGIFATAIFTLFT (137-153 of SEQ ID No. 4)	7.564906E-03
5	42	58	42	58	LAFLSFFGFFVLYSLRV (42-58 of SEQ ID No. 4)	8.236557E-02
6	328	344	328	345	GFLSAVPYLGWCWLCMI L (328-344 of SEQ ID No. 4)	.1925022
7	390	406	390	407	SLAVAFLTISTTLGGFC (390-406 of SEQ ID No. 4)	.8064944

* Relates hydrophobicity of integral sequence to the hydrophobicity of the peripheral sequence. An integral sequence with a higher hydrophobicity number is more likely to be part of a transmembrane domain.

20 A computerized alignment of the amino acid sequences of GBS toxin receptor in various organisms provides further guidance in preparing preferred fragments. See, for example, Table 4 which compares the amino acid sequence of residues 42 to 536

21.

of a human GBS toxin receptor (HP59) (residues 42 to 536 of SEQ ID NO: 8) and a sheep GBS toxin receptor (SP55).

Table 4
Alignment of Human and Sheep GBS Toxin Receptor Amino Acid

5	Sequences	
SP55	MKSPVSDLAPSDGEEGSDRTPLLRAPRAEPAPVCCSARYNLAFLSFFGF	50
HP55	MRSPVRDLARNDGEESTDRTPLLPAPRAEAPVCCSARYNLAILAFFGF	50
SP55	FVLYSLRVNLSVALVDMVDSNTTAKDNRTSYECAEHSAPIKVLHNQTGKK	100
HP55	FIVYALRVNLSVALVDMVDSNTTLEDNRTSKACPEHSAPIKVHVNQTGKK	100
SP55	YRWD AETQGWILGSFFYGYIITQIPGGYVASRSGGKLLLGFGIFATAIFT	150
HP55	YQWDAETQGWILGSFFYGYIITQIPGGYVASKIGGKMLLGFGILGTAVLT	150
SP55	LFTPLAADFGVGALVALRALEGLGEGVTYPAMHAMWSSWAPPLERSKLLS	200
HP55	LFTPIAADLGVGPLIVLRALEGLGEGVTFPAMHAMWSSWAPPLERSKLLS	200
SP55	ISYAGAQLGTVVSLPLSGVICYYMNWTVFYFFGIVGIIWFILWICLVSD	250
HP55	ISYAGAQLGTVISLPLSGIICYYMNWTVFYFFGTIGIFWFLWIVLVSD	250
SP55	TPETHKTITPYEKEYILSSLKNQLSSQKSVPWIPMLKSLPLWAIIVVAHFS	300
HP55	TPQKHKRISHYEKEYILSSLRNQLSSQKSVPWPILKSLPLWAIIVVAHFS	300
SP55	YNWTFYTLTLLPTYMKEVLRFNQENGFLSAVPYLGWLCMILSGQAAD	350
HP55	YNWTFYTLTLLPTYMKEILRFNVQENGFLSSLPYLGWLCMILSGQAAD	350
SP55	NLRARWNFSTLWVRRVFSLIGMIGPAIFLVAAGFIGCDYSLAVAFLTIST	400
HP55	NLRKWNFSTLCVRRIFSLIGMIGPAVFLVAAGFIGCDYSLAVAFLTIST	400
SP55	TLGGFCSSGFSINHLDIAPSYAGILLGITNTFATIPGMIGPIIARSLTPE	450
HP55	TLGGFCSSGFSINHLDIAPSYAGILLGITNTFATIPGMVGPVIAKSLTPD	450
SP55	NTIGEWQTVFCIAAAINVFGAIFFTLFAKGEVQNWAI SDHQHGRN	495
HP55	NTVGEWQTVFYIAAAINVFGAIFFTLFAKGEVQNWALNDHHGHRH	495
	HP55 - SEQ ID NO: 2	
	SP55 - SEQ ID NO: 4	

Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in a GBS toxin receptor sequence.

Although one class of preferred embodiments are fragments having amino- and/or carboxy-termini corresponding to amino acid positions near functional domains borders, alternative fragments may be prepared. The choice of the amino- and-carboxy-termini of such fragments rests with the discretion of the practitioner and will be made based on experimental considerations, such as ease of construction, stability to proteolysis, thermal stability, immunological reactivity, amino- or carboxyl-terminal residue modification, or other considerations. Polypeptide fragments usually contain at least nine amino acids and can contain any number of amino acids provided that the peptide fragment is at least about 80% identical to the corresponding fragment of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO:8. The human GBS toxin receptor has 41 additional amino acids on the N-terminus compared to the sheep GBS toxin receptor (compare SEQ ID NO:4 and SEQ ID NO:8). Analogs can comprise additions or deletions of some or all of those 41 N-terminal amino acids. N-terminal and C-terminal additions useful, e.g., for purification and/or antibody recognition are also contemplated. Examples include histidine tags, a FLAG (phenylalanine, leucine, alanine, guanine) epitope, fusion partners such as glutathione S transferase, chloramphenicol acetyltransferase (CAT), luciferase, β -galactosidase, and the like. Deletions of unconserved amino acids are also contemplated, provided that the structural integrity and/or binding properties of the GBS toxin receptor are not substantially compromised.

Analogs can also comprise amino acid substitutions, preferably conservative substitutions. Also preferred are conservative and/or non-conservative substitutions in regions having less shared identity among various species. For example, a variant of a GBS toxin receptor can comprise conservative and/or non-conservative substitutions of amino acids corresponding to residues 2, 6, 10, 11, 16, 17, 24, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283, 285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495 of SEQ ID NO:4. Preferably the substitution is an amino acid present in the corresponding position of SEQ ID NO:4 or SEQ ID NO:8. For example, referring to the alignment plot in Table 4, the amino acid corresponding to position 152 of SEQ ID NO:4 can be arginine (R), glutamine (Q), or a conservative or non-conservative substitution of R or Q, and preferably is R or Q. Such regions can

be identified by amino acid sequence alignment plots, such as that shown in Table 4. Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for GBS toxin, and (4) confer or modify other physicochemical or functional properties of such
5 analogs. Analogs can include various mutations of a sequence other than the naturally-occurring peptide sequence, such as, for example, single or multiple amino acid substitutions.

A conservative amino acid substitution should generally not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino
10 acid should not tend to break a helix that occurs in the parent sequence, disrupt disulfide bonds or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles*, (1984) Creighton (ed.), W.H. Freeman and Company, New York; *Introduction to Protein*
15 *Structure*, (1991), C. Branden and J. Tooze, Garland Publishing, New York, NY; and Thornton et al. (1991) *Nature* 354: 105 (which are incorporated herein by reference). A conservative substitution is a "replacement of an amino acid in a polypeptide by one with similar characteristics." (McGraw-Hill Dictionary of Scientific and
Technical Terms, Fifth Edition, 1994, Sybil P. Parker, Editor in Chief). The structure
20 and characteristics of naturally occurring amino acids has long been known in the art (Biochemistry, Second Edition, Albert L. Lehninger, 1975, pages 71-76) For example, amino acids which are similar by virtue of their hydrophobic R groups are alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, and
methionine. Alanine, valine, leucine, and isoleucine are similar by virtue of their
25 aliphatic R groups. Phenylalanine and tryptophan are similar by virtue of their aromatic R groups. Glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine are similar by virtue of their uncharged polar R groups. Glycine and alanine are similar by virtue of their small size. Serine and threonine are similar by
virtue of a hydroxyl in their R group. Asparagine and glutamine differ by only one
30 methyl group. Similarly, aspartic acid and glutamic acid differ by only one methyl group, and they are similar by virtue of their acidic R groups. Lysine, arginine, and histidine are similar by virtue of their basic R groups. In addition, lysine and arginine are similar by virtue of the amino groups on the end of the aliphatic chain in their R groups. Tyrosine and phenylalanine are similar by virtue of their aromatic groups.

Amino substitutions commonly made in the art include a substitution of valine for leucine or isoleucine, alanine for glycine, serine for threonine, asparagine for glutamine, aspartic acid for glutamic acid, and lysine for arginine, tyrosine for phenylalanine, and vice versa.

Typically, one skilled in the art would generally refrain from changing amino acids that are conserved among the various GBS toxin receptors, but a conservative substitution might reasonably be made. For example, Table 4 guides one skilled in the art to avoid substitutions, particularly nonconservative substitutions, for amino acids corresponding to residues 1, 3-5, 7-9, 12-15, 18-23, 26-30, 32-43, 45, 47-51, 54, 56-73, 76-80, 83, 85-92, 94-101, 103-131, 134-136, 138-143, 146-147, 150-154, 156-158, 160-162, 164, 167-178, 180-211, 213-218, 220-234, 237-238, 240-241, 243-245, 247-252, 255-256, 258, 261-270, 272-282, 284, 286-318, 320-323, 325-331, 334-337, 339-354, 356-361, 363-365, 367-376, 378-438, 440-441, 443-444, 446-449, 451-452, 454-460, 462-486, 489-490 and 492-494 of SEQ ID NO:4, which are conserved among the GBS toxin receptors shown in Table 4.

Tables 5 and 6 describe sequences within HP59 and SP55, respectively, that match predicted amidation, N-glycosylation, cAMP-phosphorylation, CK2-phosphorylation, myristylation (addition of unsaturated fatty acid molecules), and PKC-phosphorylation sites (Omega 1.1 sequence analysis program). The information contained in these tables provides guidance to one skilled in the art for designing GBS toxin receptor variants and fragments. When designing polypeptide variants, for example, one may decide to avoid substitutions in some or all of these regions. When designing polypeptide fragments other than immunogenic polypeptide fragments, for example, one may opt to include some or all of these regions.

Table 5 Putative Recognition Sites in HP59			Table 6 Putative Recognition Sites in SP55		
Site	Seq. ID NO: 8 Residues:	Sequence	Site	Seq. ID NO: 4 Residues:	Sequence
AMIDATION	23-26	SGRR	AMIDATION	97-100	TGKK
AMIDATION	138-141	TGKK	ASN_GLYCOSYLATION	59-62	NLSV
ASN_GLYCOSYLATION	100-103	NLSV	ASN_GLYCOSYLATION	71-74	NTTA
ASN_GLYCOSYLATION	112-115	NTTL	ASN_GLYCOSYLATION	77-80	NRTS
ASN_GLYCOSYLATION	118-121	NRTS	ASN_GLYCOSYLATION	95-98	NQTG
ASN_GLYCOSYLATION	136-139	NQTG	ASN_GLYCOSYLATION	225-228	NWTY
ASN_GLYCOSYLATION	266-269	NWTY	ASN_GLYCOSYLATION	302-305	NWTF
ASN_GLYCOSYLATION	343-346	NWTF	ASN_GLYCOSYLATION	357-360	NFST
ASN_GLYCOSYLATION	398-401	NFST	CK2_PHOSPHO_SITE	11-14	SDGE
CAMP_PHOSPHO_SITE	297-300	KRIS	CK2_PHOSPHO_SITE	73-76	TAKD
CK2_PHOSPHO_SITE	113-116	TITLE	CK2_PHOSPHO_SITE	79-82	TSYE

Table 5 Putative Recognition Sites in HP59			Table 6 Putative Recognition Sites in SP55		
Site	Seq. ID NO: 8 Residues:	Sequence	Site	Seq. ID NO: 4 Residues:	Sequence
CK2_PHOSPHO_SITE	114-117	TLED	CK2_PHOSPHO_SITE	259-262	TPYE
CK2_PHOSPHO_SITE	300-303	SHYE	CK2_PHOSPHO_SITE	452-455	TIGE
CK2_PHOSPHO_SITE	493-496	TVGE	MYRISTYL	126-131	GGYVAS
MYRISTYL	66-71	GAPRAE	MYRISTYL	142-147	GIFATA
MYRISTYL	167-172	GGYVAS	MYRISTYL	162-167	GALVAL
MYRISTYL	183-188	GILGTA	MYRISTYL	172-177	GLGEGV
MYRISTYL	213-218	GLGEGV	MYRISTYL	205-210	GAQLGT
MYRISTYL	246-251	GAQLGT	MYRISTYL	209-214	GTVVSL
MYRISTYL	250-255	GTVISL	MYRISTYL	337-342	GCWLCM
MYRISTYL	378-383	GSWLCM	MYRISTYL	386-391	GCDYSL
MYRISTYL	427-432	GCDYSL	MYRISTYL	403-408	GGFCSS
MYRISTYL	444-449	GGFCSS	MYRISTYL	423-428	GILLGI
MYRISTYL	464-469	GILLGI	MYRISTYL	427-432	GITNTF
MYRISTYL	468-473	GITNTF	PKC_PHOSPHO_SITE	17-19	SDR
PKC_PHOSPHO_SITE	23-25	SGR	PKC_PHOSPHO_SITE	37-39	SAR
PKC_PHOSPHO_SITE	58-60	TDR	PKC_PHOSPHO_SITE	55-57	SLR
PKC_PHOSPHO_SITE	78-80	SAR	PKC_PHOSPHO_SITE	73-75	TAK
PKC_PHOSPHO_SITE	120-122	TSK	PKC_PHOSPHO_SITE	97-99	TGK
PKC_PHOSPHO_SITE	138-140	TGK	PKC_PHOSPHO_SITE	254-256	THK
PKC_PHOSPHO_SITE	310-312	SLR	PKC_PHOSPHO_SITE	269-271	SLK
PKC_PHOSPHO_SITE	317-320	SQK	PKC_PHOSPHO_SITE	276-278	SQK

In light of the foregoing, preferred polypeptides comprise an amino acid sequence of the formula:

AA1-AA_n-AA_m

5 wherein:

AA1 is absent or is M;

AA_n is a contiguous chain of 0 to 100 amino acids, preferably of 0 or 41 amino acids, even more preferably of residues 2-42 of SEQ ID NO:8; and

10 AA_m is a contiguous chain of 494 amino acids comprising AA43 through AA536, wherein:

(1) each of AA43, AA47, AA51, AA52, AA57, AA58, AA65, AA66, AA72, AA85, AA87, AA93, AA94, AA96, AA115, AA116, AA122, AA123, AA125, AA134, AA143, AA173, AA174, AA178, AA185, AA186, AA189, AA190, AA196, AA200, AA204, AA206, 15 AA207, AA220, AA253, AA260, AA276, AA277, AA280, AA283, AA287, AA294, AA295, AA298, AA300, AA301, AA312, AA324, AA326, AA360, AA365, AA373, AA374, AA379, AA396, AA403, AA407, AA418, AA480, AA483, AA486, AA491, AA494, AA502, AA528, AA529, AA532 and AA536 is an essential amino acid or a 20 modified amino acid and preferably is an amino acid residue corresponding to:

(a) residue 43, 47, 51, 52, 57, 58, 65, 66, 72, 85, 87, 93, 94, 96, 115, 116, 122, 123, 125, 134, 143, 173, 174, 178, 185, 186, 189, 190, 196, 200, 204, 206, 207, 220, 253, 260, 276, 277, 280, 283, 287, 294, 295, 298, 300, 301, 312, 324, 326, 25 360, 365, 373, 374, 379, 396, 403, 407, 418, 480, 483, 486,

491, 494, 502, 528, 529, 532 and 536, respectively, of SEQ ID NO:8;

(b) residue 2, 6, 10, 11, 16, 17, 24, 25, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283, 285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495, respectively of SEQ ID NO:4; or

(c) a conservative substitution thereof;

(2) each of AA44-AA46, AA48-AA50, AA53-AA56, AA59-AA64, AA67-AA71, AA73-AA84, AA86, AA88-AA92, AA95, AA97-AA114, AA117-AA121, AA124, AA126-AA133, AA135-AA142, AA144-AA172, AA175-AA177, AA179-AA184, AA187-AA188, AA191-AA195, AA197-AA199, AA201-AA203, AA205, AA208-AA219, AA221-AA252, AA254-AA259, AA261-AA275, AA278-AA279, AA281-AA282, AA284-AA286, AA288-AA293, AA296-AA297, AA299, AA302-AA311, AA313-AA323, AA325, AA327-AA359, AA361-AA364, AA366-AA372, AA375-AA378, AA380-AA395, AA397-AA402, AA404-AA406, AA408-AA417, AA419-AA478, AA481-AA482, AA484-AA485, AA487-AA490, AA492-AA493, AA495-AA501, AA503-AA527, AA530-AA531 and AA533-AA535 is

(a) residue 44-46, 48-50, 53-56, 59-64, 67-71, 73-84, 86, 88-92, 95, 97-114, 117-121, 124, 126-133, 135-142, 144-172, 175-177, 179-184, 187-188, 191-195, 197-199, 201-203, 205, 208-219, 221-252, 254-259, 261-275, 278-279, 281-282, 284-286, 288-293, 296-297, 299, 302-311, 313-323, 325, 327-359, 361-364, 366-372, 375-378, 380-395, 397-402, 404-406, 408-417, 419-478, 481-482, 484-485, 487-490, 492-493, 495-501, 503-527, 530-531 and 533-535, respectively, of SEQ ID NO:8; or

(b) a conservative substitutions thereof; and

(3) AA315 through AA367 are optionally absent.

Preferred polypeptides comprise the amino acid sequence of SEQ ID NO:4, SEQ ID NO:8 or an amino acid sequence which varies from that sequence only at the specific residues which are not conserved between the sheep GBS toxin receptor (SEQ ID NO:4) and the human GBS toxin receptor (SEQ ID NO:8). Of those variations, the most preferred variations are those resulting in a polypeptide encoded by SEQ ID NO:11. Even more preferred variations are those amino acids in the corresponding positions of the amino acid sequence of SEQ ID NO:4. Particularly preferred are polypeptides comprising an amino acid sequence that differs from SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:8 at no more than about 20% of the amino acid residues, with increasing preference for no more than about 10%, 5%, 1%, with one to zero amino acid differences being most preferred.

Besides targeting specific amino acids for change, analogs of GBS toxin receptor can also be prepared by techniques involving activity selection, such as, for example, phage display, directed evolution, DNA shuffling, and homologous in procaryotes or eucaryotes of genes from different species, as described in part in U.S. Patent Nos. 5,605,793; 5,830,721; 5,811,238; 5,837,458; 5,093,257; 5,223,409; 5,403,484; 5,571,698; and 5,837,500, which are incorporated herein by reference.

Any variant or fragment of the human and sheep GBS toxin receptors described herein can be tested for the requisite activity by determining whether the variant or fragment can bind GBS toxin.

10 These polypeptides provide reagents useful in drug discovery and purification and can be used in various *in vitro* assays, preferably when expressed on the surface of a cell, e.g., a stable transfected cell. For example, assays such as binding assays can be used to screen test compounds, including polysaccharides and other compounds, for their ability to bind the GBS toxin receptor. Assays can identify
15 potential drug candidates that block GBS toxin binding to the GBS toxin receptor. Such drugs are useful for preventing and/or treating early onset disease in neonatal humans. Some polypeptides can be used to competitively inhibit binding GBS toxin to a GBS toxin receptor.

The polypeptides of the invention can be used to affinity purify GBS toxin, a GBS toxin chimeric compound, and other polysaccharides or compounds which can bind the GBS toxin receptor.

The polypeptides can also be used to develop a method of targeting a cytotoxic agent for delivery to a cell that expresses a GBS toxin receptor. For example, a cytotoxic agent can be coupled to a molecule that binds a GBS toxin receptor for selective delivery to the neovasculature of a growing tumor. Such a delivery system would permit a highly concentrated, localized attack on a growing tumor, while minimizing the adverse systemic side effects encountered with most chemotherapeutics. In one instance, the cytotoxic agent can be GBS toxin, which, upon binding to GBS toxin receptor, induces an inflammatory response as described in Hellerqvist et al., Angiogenesis: Molecular Biology, Clinical Aspects, Edited by M.E. Maragoudakis et al., Plenum Press, New York 1994, pp. 265-269. In a similar manner, selective delivery of a therapeutic agent to a cell that expresses a GBS toxin receptor could be used advantageously to treat tumors, rheumatoid arthritis or neural injury, or to facilitate wound healing.

The polypeptides of the invention can also be used to screen for and/or design a GBS toxin mimetic with improved therapeutic properties, such as, for example, improved ability to inhibit hypoxia-induced neovascularization or angiogenesis. Such mimetics are useful in the treatment and prevention of conditions resulting from hypoxia-induced neovascularization or angiogenesis, such as, for example, tumor growth, scarring during wound healing, gliosis during repair of neural injury, reperfusion injury, restenosis, rheumatoid arthritis, psoriasis, other chronic inflammatory diseases characterized by angiogenesis, etc. Therapeutic properties can be improved by enhancing biological stability, affinity for the GBS toxin receptor, complement binding activity, reducing antigenicity, etc.

The polypeptides of the invention can also be used to generate antibodies for various therapeutic and research purposes. The polypeptides of the invention can be used to immunize rabbits, mice, goats, chickens, or other animals known in the art to be amenable to such immunization. Monoclonal antibodies are generally preferred but polyclonal antibodies can also be used, provided that detection of binding of the GBS toxin receptor antibody to the GBS toxin receptor is possible. The production of non-human monoclonal antibodies, e.g., murine, is well known (see, e.g., Harlow et al., *Antibodies A Laboratory Manual*, Cold Spring Harbor Press, pp. 139-240, 1989,

incorporated herein by reference). As it may be difficult to generate human monoclonal antibodies to a human receptor or binding domain polypeptide, it may be desirable to transfer antigen binding regions of non-human monoclonal antibodies, e.g. the F(ab')₂ or hypervariable regions or murine monoclonal antibodies, to human constant regions (Fc) or framework regions by recombinant DNA techniques to produce substantially human molecules. Such methods are generally known and are described in, e.g., U.S. Pat. Nos. 4,816,397 and 4,946,778, and EP publications 173,494 and 239,400. Alternatively, one may isolate DNA sequences which code for a human monoclonal antibody or portions thereof that specifically bind to the receptor protein by screening a DNA library from human B cells according to the general protocol outlined in WO 90/14430, and then cloning and amplifying the sequences which encode the antibody (or binding fragment) of the desired specificity.

Usually, polypeptides used for producing antibodies are the full-length receptor or receptor fragments designed from putative extracellular domains identified by a variety of methods known in the art, including computer programs which predict secondary and tertiary structure of a polypeptide based upon its primary amino acid sequence. Another method for designing antigenic peptides utilizes computer programs that predict the high points of hydrophilicity within a particular primary amino acid sequence. For example, using the method of Happ and Woods, *Proc. Natl. Acad. Sci. USA* (1981) 78:3824-3829, via the "Antigen" program in PC/GENE, the inventors identified 3 regions of high hydrophilicity, shown below in Table 7, and used the results to design antigenic peptides to be used in the preparation of antibodies against GBS toxin receptor (see Example 4).

Table 7

High Points of Hydrophilicity in SP55

No.	Ah	Sequence
1	2.05	Glu-Glu-Gly-Ser-Asp-Arg (14-19 of SEQ ID No. 2)
2	1.52	Lys-Asp-Asn-Arg-Thr-Ser (75-80 of SEQ ID No. 2)
3	1.33	Arg-Ala-Pro-Arg-Ala-Glu (25-30 of SEQ ID No. 2)

Ah = Average hydrophilicity.

Antibodies that recognize various portions of the intact GBS toxin receptor can be used to further investigate structure and function of the receptor. The polypeptides of the invention can give rise to antibodies that recognize a variety of forms of GBS toxin receptor, including, but not limited to, intact GBS toxin receptor

expressed on a cell surface, denatured GBS toxin receptor or non-denatured GBS toxin receptor, and GBS toxin receptor purified away from cellular components or GBS toxin receptor contained in a cell lysate. GBS toxin receptor antibodies can be used to study species differences as well as GBS toxin receptor expression levels in various cell types.

Antibodies that recognize a portion or all of an extracellular domain are particularly useful as a diagnostic for the monitoring of tumor growth and metastasis, for the detection or identification of a chronic inflammatory condition, such as, for example, rheumatoid arthritis or psoriasis, and for the detection of other medical conditions arising due to hypoxia-driven angiogenesis, such as, for example, restenosis. Typically, such antibodies can be employed in a variety of standard research and diagnostic techniques, including, but not limited to, western blot, immunoprecipitation, ELISA, radioimmunoassay (RIA), BIACOR®, enzyme-linked-immunoassay (EIA), immunofluorescence, fluorescence activated cell sorting (FACS), and *in vivo* diagnostic imaging systems such as magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR), computerized axial tomography (CAT) scan, and position emission tomography (PET), etc.

In addition, antibodies that block the binding of GBS toxin to a GBS toxin receptor can be used for the treatment or prevention of early onset disease in a neonatal human. Such antibodies can directly or indirectly block the GBS toxin binding site on the GBS toxin receptor.

In one embodiment, the GBS toxin receptor protein is naturally occurring and can be isolated from a cell extract by protein purification techniques known in the art, such as, for example, ion exchange column chromatography, high performance liquid chromatography (HPLC), reversed phase HPLC, or affinity chromatography using antibodies that recognize the GBS toxin receptor.

Alternatively, the isolated proteins and polypeptides are expressed using polynucleotides encoding the polypeptide(s) of the invention in operative association with an appropriate control sequence including a promoter in an expression vector suitable for expression, preferably in a mammalian cell, and also in bacterial, insect, or yeast cells.

Usually, the GBS toxin receptor polynucleotide or a fragment thereof can be expressed in a mammalian system. Such expression will usually depend on a mammalian promoter, which is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. Usually, a promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region typically includes an RNA polymerase binding site and a transcription initiation site.

Vectors suitable for replication in mammalian cells are known in the art, and can include viral replicons, or sequences that ensure integration of the sequence encoding PAK65 into the host genome. Suitable vectors can include, for example, those derived from simian virus SV40, retroviruses, bovine papilloma virus, vaccinia virus, and adenovirus.

A suitable vector, for example, is one derived from vaccinia viruses. In this case, the heterologous DNA is inserted into the vaccinia genome. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art, and utilize, for example, homologous recombination. The insertion of the heterologous DNA is generally into a gene which is non-essential in nature, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid shuttle vectors that greatly facilitate the construction of recombinant viruses have been described (see, for example, Mackett et al. (1984); Chakrabarti et al. (1985); Moss (1987)). Expression of the heterologous polypeptide then occurs in cells or individuals which are immunized with the live recombinant vaccinia virus.

Such suitable mammalian expression vectors usually contain one or more eukaryotic transcription units that are capable of facilitating expression in mammalian cells. The transcription unit is comprised of at least a promoter element to mediate transcription of foreign DNA sequences. Suitable promoters for mammalian cells are known in the art and include viral promoters such as those from simian virus 40 (SV40) (Subramani et al., Mol Cell. Biol. 1:854-864, 1981), cytomegalovirus (CMV) (Boshart et al., Cell 41:521-530, 1985), Rous sarcoma virus (RSV), adenovirus (ADV) (Kaufman and Sharp, Mol. Cell. Biol. 2:1304-1319, 1982), and bovine papilloma virus (BPV), as well as cellular promoters, such as a mouse metallothionein-1 promoter (U.S. Patent No. 4,579,821), a mouse VK promoter (Bergman et al., Proc. Natl. Acad. Sci. USA 81:7041-7045, 1993; Grant et al., Nuc. Acids Res. 15:5496, 1987), and a mouse VH promoter (Loh et al., Cell 33:85-93, 1983).

The optional presence of an enhancer element (enhancer), combined with the promoter elements described herein, will typically increase expression levels. An enhancer is any regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to endogenous or heterologous promoters, with synthesis beginning at the normal mRNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or

flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.). Enhancer elements derived from viruses can be particularly useful, because they typically have a broader host range. Examples useful in mammalian
5 cells include the SV40 early gene enhancer (Dijkema et al (1985) *EMBO J.* 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) *Proc. Natl. Acad. Sci.* 79:6777), from human cytomegalovirus (Boshart et al. (1985) *Cell* 41:521) as well as the mouse μ enhancer (Gillies, *Cell* 33:717-728, 1983). Additionally, some enhancers are regulatable and
10 become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237).

In addition, the transcription unit can also be comprised of a termination sequence and a polyadenylation signal which are operably linked to the GBS toxin
15 receptor coding sequence. Polyadenylation signals include, but are not limited to, the early or late polyadenylation signals from SV40 (Kaufman and Sharp), the polyadenylation signal from the Adenovirus 5 E1B region and the human growth hormone gene terminator (DeNoto et al., *Nuc. Acids Res.* 9:3719-3730, 1981).

Sequences that cause amplification of the gene may also be desirable, as are
20 sequences which encode selectable markers. Selectable markers for mammalian cells are known in the art, and include, for example, thymidine kinase, dihydrofolate reductase (together with methotrexate as a DHFR amplifier), aminoglycoside phosphotransferase, hygromycin B phosphotransferase, asparagine synthetase, adenosine deaminase, and antibiotic resistant genes such as neomycin.

25 A GBS toxin receptor, or fragment thereof, can be expressed on the surface of a cell, or can be expressed in soluble or secreted form. Expression on the surface of the cell can be achieved, for example, by including a secretory leader operably linked to a nucleic acid sequence encoding the desired receptor fragment and at least one transmembrane domain. The secretory leader can be that encoded by the GBS toxin
30 receptor gene, or can be a heterologous leader sequence commonly used in the art, such as, for example, the leader sequence of *Schizosaccharomyces pombe* *pho1+* acid phosphatase (Braspenning et al., *Biochem Biophys Res. Commun* (1998) 245:166-71), the leader sequence of human interleukin-2 (IL-2) gene (Sasada et al., *Cell Struct*

Funct (1988) 13:129-141). Expression in soluble or secreted form can be achieved, for example, by excluding from the gene construct nucleic acid sequences encoding a transmembrane domain. In some instances, solubility and/or secretion are achieved by the use of a fusion partner, such as, for example, chloramphenicol acetyltransferase (CAT), β -galactosidase, and other genes readily expressed in the selected host cell.

The vector that encodes GBS toxin receptor can be used for transformation of a suitable mammalian host cell. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (these patents are incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), N1E-115 (Liles et al., J. Biol. Chem. 261:5307-5313, 1986), PC 12 human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines, such as insect derived cell lines IF9 and IF21. Cell lines of particular preference are those expressing recombinant GBS toxin receptor constructs constitutively, lines which subsequently develop characteristics of a transformed cell, and lines which more preferably express GBS toxin receptor or fragments on the cell surface. Particularly preferred are ECV cells (a bladder carcinoma cell line originally referred to in the scientific literature as an endothelial cell line), human umbilical vein endothelial cells (HUVEC), bovine, sheep, and human adrenal medulla endothelial cells.

Recombinant GBS toxin receptor or fragments thereof can be produced by culturing host cells expressing the receptor or fragment in a suitable culture medium and under appropriate cell culture conditions. Culture media and conditions are

variable depending on the requirements of a particular host cell line and are well known in the art. Typically, cells are cultured at 37°C in a cell culture incubator with a fixed amount of CO₂, usually in the range of 5-10%.

In another embodiment, the polypeptide fragments can be synthesized chemically by techniques well known in the art, such as solid-phase peptide synthesis (Stewart et al., Solid Phase Peptide Synthesis, W.H. Freeman Co., San Francisco (1963)); Merrifield, J Am Chem Soc 85:2149-2154 (1963)). These and other methods of peptide synthesis are also exemplified by U.S. Patent Nos. 3,862,925, 3,842,067, 3,972,859, and 4,105,602. The synthesis can use manual synthesis techniques or automatically employ, for example, an Applied BioSystems 430A or 431A Peptide Synthesizer (Foster City, California) following the instructions provided in the instruction manual supplied by the manufacturer. It will be readily appreciated by those having ordinary skill in the art of peptide synthesis that the intermediates which are constructed during the course of synthesizing the present analog compounds are themselves novel and useful compounds and are thus within the scope of the invention.

In addition to polypeptides consisting only of naturally-occurring amino acids, peptidomimetics are also provided. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (Fauchere, J. (1986) Adv. Drug Res. 15: 29; Veber and Freidinger (1985) TINS p.392; and Evans et al. (1987) J. Med. Chem 30: 1229, which are incorporated herein by reference) and are usually developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity) but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: -CH₂NH-, -CH₂S-, -CH₂-CH₂-, -CH=CH- (cis and trans), -COCH₂-, -CH(OH)CH₂-, and -CH₂SO-, by methods known in the art and further described in the following references: Spatola, A.F. in "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Morley, J.S., Trends Pharm Sci (1980) pp. 463-468 (general

review); Hudson, D. et al., *Int J Pept Prot Res* (1979) 14:177-185 (-CH₂NH-, CH₂CH₂-); Spatola, A.F. et al., *Life Sci* (1986) 38:1243-1249 (-CH₂-S); Hann, M.M., *J Chem Soc Perkin Trans I* (1982) 307-314 (-CH-CH-, cis and trans); Almqvist, R.G. et al., *J. Med Chem* (1980) 23:1392-1398 (-COCH₂-); Jennings-
5 White, C. et al., *Tetrahedron Lett* (1982) 23:2533 (-COCH₂-); Szelke, M. et al., *European Appln. EP 45665* (1982) CA: 97:39405 (1982) (-CH(OH)CH₂-); Holladay, M.W. et al., *Tetrahedron Lett* (1983) 24:4401-4404 (-C(OH)CH₂-); and Hruby, V.J., *Life Sci* (1982) 31:189-199 (-CH₂-S-); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is -CH₂NH-. Such peptide
10 mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others. Labeling of peptidomimetics usually involves covalent attachment of one or
15 more labels, directly or through a spacer (e.g., an amide group), to non-interfering position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with GBS toxin (e.g., are not contact points in the GBS toxin binding domain of the GBS toxin receptor). Derivatization (e.g.,
20 labelling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic.

Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a
25 consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch (1992) *Ann. Rev. Biochem.* 61: 387, incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

30 The invention also provides a complex comprising a GBS toxin bound to a mammalian GBS toxin receptor or a fragment of a mammalian GBS toxin receptor. Preferably, the complex comprises a GBS toxin bound to a GBS toxin receptor polypeptide described above that can bind GBS toxin. Typically, a complex is formed by contacting a GBS toxin with such a polypeptide under conditions that

permit specific binding of the GBS toxin to the polypeptide. The GBS toxin can be labeled or unlabeled. The polypeptide can be present on the surface of a cell, or immobilized in a well or on a bead, or the polypeptide can be present in solution.

5 DETECTION METHODS

Yet another aspect of the invention provides methods for detecting or monitoring a variety of medical conditions characterized by pathologic and/or hypoxia-driven angiogenesis or neovascularization. Examples include, but are not limited to, early onset disease in the neonate, and the progression of cancers involving
10 tumors.

Early onset disease can be diagnosed by detecting the presence or absence of GBS toxin in a patient. One method of detection is a competition assay that determines the effect of a suspected sample on the formation of a complex between GBS toxin and a GBS toxin receptor or fragment thereof. For example, the method
15 comprises contacting a control GBS toxin with a GBS toxin receptor polypeptide, in the presence and absence of a sample suspected of containing GBS toxin and under conditions that permit specific binding of the GBS toxin to the polypeptide, and comparing the amount of complex formation achieved in the presence of the suspected sample to the amount of complex formation achieved in the absence of the
20 suspected sample. Preferably, the control GBS toxin is substantially purified and of a known concentration. Preferably, the control GBS toxin further comprises a label. Suitable labels include, but are not limited to, radioisotopes, chromophores, fluorophores, biotin, avidin, and other labels used by one skilled in the art. Another method directly measures, rather than by competition with a control GBS toxin,
25 complex formation between GBS toxin present in a suspected sample and a GBS toxin receptor polypeptide.

Pathologic vasculature can be detected in a mammalian tissue by detecting the presence or absence of GBS toxin receptor in the region of a tumor, with the presence of GBS toxin receptor being indicative of the presence of pathologic vasculature. The
30 method can be used to monitor tumor growth or metastasis. One method of detection involves the use of molecules, e.g. antibodies, that specifically bind to a GBS toxin receptor, preferably an extracellular domain of GBS toxin receptor. Typically, the method comprises administering, to a mammalian tissue, e.g. in a mammal having a cancerous tumor, e.g., an antibody that recognizes a GBS toxin receptor, and

detecting specific binding of the antibody. Typically, the antibody is a labeled antibody. Preferably, the observations are quantitative and can be visual.

During surgery, the margin of a tumor can be visualized by any of a number of imaging techniques known in the art and described above. The imaging of the tumor is effected by detecting the binding of a labeled antibody or other molecules to the GBS toxin receptor on the pathologic vasculature of a tumor. This type of surgery is also known as virtual surgery because while performing the surgery, the surgeon views the tumor indirectly on an imaging screen.

10 DRUG DISCOVERY

A fourth aspect of the invention provides methods, using the polypeptides of the invention, of identifying drug candidates for the treatment of medical conditions characterized by hypoxia-driven angiogenesis or neovascularization. Preferred compounds are competitive inhibitors of GBS toxin binding to a GBS toxin receptor or inhibit GBS toxin receptor activity. Particularly preferred are compounds that inhibit the first phosphorylation step in the signal transduction pathway. Compounds can be produced by a variety of random drug design methods commonly known in the art, such as, for example, combinatorial chemistry (U.S. Patent No. 5,646,285; U.S. Patent No. 5,639,603), peptide libraries (U.S. Patent No. 5,591,646; U.S. Patent No. 5,367,053; U.S. Patent No. 5,747,334), phage display (U.S. Patent No. 5,403,484; U.S. Patent No. 5,223,409), SELEX® (U.S. Patent No. 5,773,598; U.S. Patent No. 5,763,595; U.S. Patent No. 5,763,566), and combinatorial carbohydrate chemistry (Hirschmann et al., J Med Chem (1996) 39:2441-2448; Hirschmann et al., J Med Chem (1998) 41:1382-1391; Sofia MJ, Mol Divers (1998) 3:75-94; U.S. Patent No. 5,780,603; U.S. Patent No. 5,756,712)

An alternative approach is rational drug design with the intent of producing a GBS toxin mimetic or a GBS toxin receptor mimetic with improved therapeutic properties using techniques such as x-ray crystallography, nuclear magnetic resonance (NMR) correlation spectra (U.S. Patent No. 5,698,401), computer assisted molecular modeling (U.S. Patent No. 5,579,250; U.S. Patent No. 5,612,895; U.S. Patent No. 5,680,331, Cooper et al., J. Comput.-Aided Mol. Design, 3:253-259 (1989); Brent et al., J. Comput.-Aided Mol. Design 2:311-310 (1988)) and other methods of rational drug design known in the art. FIG. 1 provides a broad overview of some of the main steps in some of the rational drug design methods of the present invention. For

example, one approach to rational drug design involves a computer program, such as INSIGHTII (available from Bisoym Technologies, 10065 Barnes Canyon Road, San Diego, California) to identify active sites in proteins by homology-based modeling. This method facilitates the modeling of a protein by using a similar protein whose
5 structure is well known. Commercial software containing search algorithms for three dimensional database comparisons are available from vendors such as Day Light Information Systems, Inc., Irvine, California 92714, and Molecular Design Limited, 2132 Faralton Drive, San Leandro, California 94577.

In one embodiment, the compound can bind the GBS toxin receptor and
10 induce an inflammatory response in a manner similar to the binding of GBS toxin to the GBS toxin receptor. Such compounds can be used, for example, as a drug to target an inflammatory response to the developing vasculature of a tumor.

In another embodiment, the compound can bind the GBS toxin receptor with or without inducing an inflammatory response, preferably without inducing an
15 inflammatory response. In one instance, the compound can be used as a vehicle to target pathological neovasculature for treatment with a cytotoxic agent. For example, the cytotoxic agent can be chemically coupled to the compound to form a chimeric drug. Such chimeric drugs can be used in the treatment of tumors, rheumatoid arthritis, wound healing, spinal cord injury, and other conditions characterized by
20 hypoxia-driven angiogenesis or neovascularization. In another instance, the compound can be used directly to competitively inhibit binding of GBS toxin to a GBS toxin receptor. Such compounds can be used in the treatment of early-onset disease in the neonate.

In a third embodiment, the compound can bind GBS toxin and can be used in
25 the treatment of early-onset disease in the neonate.

The polynucleotides of the invention can be expressed in random mutagenesis systems such as phage display or the yeast two-hybrid system for the synthesis and identification of mutant peptide GBS toxin receptor polypeptides that bind GBS toxin. Alternatively, immobilized or soluble GBS toxin receptor fragments of the invention
30 can be used to screen combinatorial peptide and combinatorial chemical libraries and non-random recombinant and synthetic peptides and other compounds (such as non-peptide molecules) for GBS toxin receptor binding. Compounds that bind GBS toxin or GBS toxin receptor can then be further characterized in a functional assay for any of the activities described above in order to identify a drug candidate for the treatment

of medical conditions involving angiogenesis or neovascularization.

A compound which inhibits binding of GBS toxin to a GBS toxin receptor can be identified by combining a test compound with a mammalian GBS toxin receptor or fragment thereof capable of binding GBS toxin, under conditions that permit specific
5 binding of GBS toxin to the GBS toxin receptor or fragment, and determining the amount of inhibition by the compound of the binding of GBS toxin to the GBS toxin receptor or fragment.

In a preferred embodiment, the GBS toxin receptor or fragment is expressed by a cell, preferably on the cell surface. The cells are contacted with labeled GBS
10 toxin in the presence or absence of the test compound. A change in the binding of GBS toxin to the GBS toxin receptor is then determined. Alternatively, the GBS toxin is unlabeled and an antibody that recognizes GBS toxin is labeled instead. The labeled antibody is used to measure inhibition by a compound of GBS toxin binding to the GBS toxin receptor or fragment. In another embodiment, the GBS toxin
15 receptor or fragment is not associated with a cell, but is instead coupled to a matrix, such as, for example, a well in a microtiter plate or a bead. Additional suitable solid supports include latex, polystyrene beads (Interfacial Dynamics Corp. Portland, Oreg.), magnetic particles (Advanced Magnetics, Cambridge, Mass.) and nylon balls (Hendry et al., *J. Immunological Meth.*, 35:285-296, 1980). The receptor or fragment
20 can be coupled to the matrix directly or indirectly through an antibody, coupled to the matrix, that binds the receptor fragment. In a third embodiment, the GBS toxin receptor or fragment is soluble and can be immunoprecipitated with an antibody that recognizes the receptor or fragment.

A preferred method for identifying a compound which binds a mammalian
25 GBS toxin receptor comprises the steps of (1) combining a test compound with a GBS toxin receptor or fragment thereof under conditions that allow specific binding to occur, and (2) detecting a complex formed between the test compound and the GBS toxin receptor or fragment. A preferred method is a competition assay which determines the ability of the test compound to compete for binding to the GBS toxin
30 receptor or fragment. In such an assay, GBS toxin is combined with the GBS toxin receptor or fragment in the presence or absence of the test compound. Decreased specific binding of GBS toxin in the presence versus the absence of the test compound is indicative of the ability of the test compound to bind a mammalian GBS toxin receptor. Another method comprises combining a control compound with the GBS

toxin receptor or fragment under the same conditions as the test compound and comparing the amount of complex formation between the test compound or the control compound and the GBS toxin receptor or fragment thereof. Preferably, the test compound and/or the control compound are labeled. The test compound can be

5 any of a number of classes of compounds, such as for example, small organic molecules (such as those used for and obtained by combinatorial chemistry), polysaccharides, polypeptides, RNA, antibodies, and single chain antibodies. In a preferred embodiment, the polypeptide is expressed by a cell, preferably on the surface of the cell, and preferably by a stable transfected cell. Such a system is

10 particularly useful for testing the effectiveness of a chimeric compound comprising a cytotoxic agent. The cytotoxic activity of the compound can be determined by exposing a cell expressing the GBS toxin receptor on the cell surface to the test chimeric compound and detecting signs of cytotoxicity. One could detect such signs by a viability stain of the cell, by detecting apoptosis (for example, by a DNA ladder

15 assay or a TUNEL™ stain, which binds to broken DNA), by measuring tritiated thymidine incorporation into the cell, and by quantitating kinase-dependent phosphorylation (e.g., using phosphoantibodies or various phosphoimaging techniques).

In another embodiment, the invention provides a method for identifying an

20 inhibitor of GBS toxin receptor. The method comprises incubating test cells in the presence and absence of a test compound. The test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity (e.g., a fragment that increases the proliferation or migration of the expressing cells relative to control cells of the same cell type that do not express the fragment). The test cells are incubated

25 under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate. Control cells that do not express the GBS toxin receptor or fragment proliferate or migrate less than cells that express the GBS toxin receptor or fragment. The proliferation or migration (also referred to herein as motility) of the test cells incubated in the presence or absence of the test compound is compared. Less

30 proliferation or migration in the presence of the test compound than in the absence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor. Preferably, as a control to determine whether the test compound specifically inhibits the GBS toxin receptor, the proliferation or migration of control cells in the presence and absence of the test compound is also compared. In the

- absence of a difference in the proliferation or migration of control cells incubated in the presence or absence of the test compound, decreased proliferation or migration in test cells exposed to the test compound relative to test cells not exposed to the test compound is indicative of specific inhibition of the GBS toxin receptor. It will be readily apparent that the control portions of the method need not be performed contemporaneously with the test portions of the method. For example, control cells can be incubated with a battery of test compounds to determine cellular effects of the test compounds prior to incubating the test cells with the test compounds. Motility or migration can be determined by detecting movement of cells on a culture dish.
- 5 Proliferation can be detected in a number of ways, including, but not limited to, measuring tritiated thymidine incorporation, cell counts, apoptosis assays, and viability assays. Preferred cells include cells transfected with GBS toxin receptor, preferably endothelial cells transfected with GBS toxin receptor, even more preferably vascular endothelial cells or microvascular endothelial cells. Primary cells that express GBS toxin receptor are also preferred, for example, endothelial cells that have been passaged in cell culture, at confluence, no more than 8 or 9 times. A preferred class of test compounds includes kinase inhibitors, preferably cAMP-dependent kinase inhibitors, PKC inhibitors, and CK2 inhibitors, which can be used as a starting point for developing more specific GBS toxin receptor inhibitors.
- 10 Another class of compounds includes antibodies specific for GBS toxin receptor. Particularly preferred are single chain antibodies, preferably a collection of single chain antibodies that recognize various epitopes on the GBS toxin receptor. Less preferred are divalent antibodies specific for the binding site of the GBS toxin receptor ligand because they may trigger the signal transduction cascade upon dimerization.
- 15 20 25

Another embodiment of the invention is a method of identifying an inhibitor of endothelial cell proliferation or migration, which are essential components of angiogenesis. The method basically comprises the steps described in the preceding paragraph and uses endothelial cells.

- 30 Yet another embodiment of the invention is a method of identifying a therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic or hypoxia-driven angiogenesis or neovascularization. The method basically comprises the steps described above and uses cells from tissues derived from mammals afflicted with the medical condition or cells that serve as a

model for afflicted tissue.

A preferred method for designing a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor comprises (1) simulating and selecting the most probable conformations of a GBS toxin receptor or fragment thereof, (2) designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the GBS toxin receptor or fragment, (3) chemically synthesizing the analog, and (4) evaluating the bioactivity of the analog. Preferably, steps (a) and (b) are performed with the aid of a computer program.

10 A preferred method for designing a compound which binds to a mammalian GBS toxin receptor comprises (1) simulating and selecting the most probable conformations of a GBS toxin receptor or fragment thereof, (2) deducing most probable binding domains of the receptor or fragment, (3) designing a compound that would form the energetically most probable complexes with the receptor or fragment, 15 (4) chemically synthesizing the compound, and (5) evaluating the bioactivity of the compound. Preferably, steps (a)-(c) are performed with the aid of a computer program.

Preferred polypeptides for use in the screening assays described above are polypeptides sharing at least about 85% identity, preferably at least about 95% 20 identity, and most preferably greater than about 99% identity with the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment thereof having GBS toxin receptor activity. Most preferred are polypeptides having an amino acid sequence of SED ID NO: 2, 4 OR 8 or a fragment thereof having GBS toxin receptor activity.

25 METHODS OF PURIFICATION

Another aspect of the invention is a method for purifying a compound that binds a GBS toxin receptor, for example, natural ligand, other polysaccharides, or an antibody specific for the GBS toxin receptor. The method comprises providing a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that 30 binds GBS toxin, contacting the polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide, and separating the bound compound from the remainder of the sample. The polypeptide can be soluble but preferably is immobilized on a substrate e.g., on a bead, membrane or on the surface of a cell, preferably a stable transfected cell.

METHODS OF TREATMENT

GBS toxin receptor polypeptides and antibodies that interfere with GBS toxin binding can be used in a method of treatment of the human or animal body. For example, such inhibitors of GBS toxin binding can be administered to a patient to

5 treat or prevent medical conditions involving GBS toxin binding to a GBS toxin receptor, such as, for example, early onset disease in the neonate.

GBS toxin mimetics or other compounds that bind and/or inhibit GBS toxin receptor, some of which can be identified by the drug discovery assays of the invention, can be used in a method of treatment of the human or animal body or can be used for the manufacture of a medicament for the treatment or prevention of any of a number of medical conditions involving pathologic and/or hypoxia-driven angiogenesis, such as, for example, cancerous tumors, chronic inflammatory diseases, scarring during wound healing or repair of neural injury.

In a preferred embodiment, such a compound exerts its therapeutic effect by binding GBS toxin receptor and evoking an inflammatory response, as does GBS toxin. Preferably, such compounds comprise a sulfhydryl, hydroxyl, or amino group displayed so as to be available for binding complement C3.

In another preferred embodiment, the compound is an inhibitor of GBS toxin activity. Preferred inhibitors include, but are not limited to, kinase inhibitors, single chain antibodies specific for the GBS toxin receptor, and antisense polynucleotides that specifically hybridize under high stringency conditions to a GBS toxin receptor nucleic acid sequence, such as that of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:7.

In another preferred embodiment, the compound exerts its therapeutic effect without evoking an inflammatory response. The compound can be used to deliver a cytotoxic agent to tissue in close proximity to a cell expressing a GBS toxin receptor, such as, for example, a tumor undergoing angiogenesis. Preferably, the compound is covalently attached to a cytotoxic agent and can be associated non-covalently with a cytotoxic agent, such as, for example, on the external surface of a liposome, micelle, or other lipophilic drug encapsulating structure. Preferred cytotoxic agents include antineoplastic agents commonly known in the art, such as, for example, mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide, and other alkylating agents, methotrexate and other folate antagonists, 6-mercaptopurine and other purine antagonists, 5-fluorouracil and other pyrimidine antagonists, cytarabine, ovinblastine, vincustine, and other vincas, etoposide and other podophyllotoxins, doxorubicin, bleomycin, mitomycin, and other antibiotics, carmustine, lomustine and other nitrosureas, cisplatin, interferon, asparaginase, tamoxifen, flutamide, and taxol. Other preferred biologic agents include sense and/or antisense RNA or DNA sequences derived from specific tumor promoter or suppressor genes, such as, for example, the p53 and TGF gene families, signal transduction protein family members such as, for example, ras and myc, and growth factor receptor kinases such as, for

example flt2 and flk1, Tai1, Tai2, and neuropholin, and other genes implicated in neoplastic disease and other diseases driven by pathologic angiogenesis.

In another embodiment, GBS toxin receptor polypeptide or fragment thereof can be administered to a subject as a decoy to reduce the amount of stimulation of the GBS toxin receptor present in afflicted tissues (e.g., tumor tissues), thereby reducing cellular responses leading to proliferation and migration of cells of the afflicted tissues. Preferably, the GBS toxin receptor polypeptide or fragment is administered in soluble form, even more preferably sans transmembrane domains.

10 PHARMACEUTICAL COMPOSITIONS

Polypeptides of the invention that comprise a domain essential for GBS toxin binding that have the desired characteristics for bioavailability, stability and other important parameters of pharmacokinetics *in vivo* can be used as a competitive inhibitor of GBS toxin binding for medical conditions, such as, for example, early onset disease in the neonate, in which GBS toxin binding is undesirable. Appropriate polypeptides can include fragments having an amino acid sequence corresponding to a partial or full sequence of SEQ ID NO: 2 or SEQ ID NO: 4 or analogs thereof.

Compounds determined by assays using the polypeptides of the invention to bind and/or GBS toxin receptor and/or induce an inflammatory response, and that have the desired pharmacokinetic characteristics, can be used as treatments for medical conditions in which GBS toxin binding can be therapeutic, such as, for example, medical conditions involving pathologic or hypoxia-driven angiogenesis or neovascularization.

Pharmaceutical compositions of the invention include a pharmaceutically acceptable carrier that may contain a variety of components that provide a variety of functions, including regulation of drug concentration, regulation of solubility, chemical stabilization, regulation of viscosity, absorption enhancement, regulation of pH, and the like. For example, in water soluble formulations the pharmaceutical composition preferably includes a buffer such as a phosphate buffer, or other organic acid salt, preferably at a pH of between about 7 and 8. Other components may include antioxidants, such as ascorbic acid, hydrophilic polymers, such as, monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, dextrans, chelating agents, such as EDTA, and like components well

known to those in the pharmaceutical sciences, e.g. Remington's Pharmaceutical Science, latest edition (Mack Publishing Company, Easton, PA).

An effective amount of an active compound such as a GBS toxin receptor polypeptide, mimetic or analog, or GBS toxin mimetic or analog for particular applications depends on several factors, including the chemical nature of the polypeptide, mimetic or analog, the disorder being treated, the method of administration, and the like. Preferably, an effective amount will provide a concentration of polypeptide or mimetic of between about 0.0001 to 100 μM at the target GBS toxin receptor on a cell surface, more preferably less than 10 μM , with less than 1 μM being most preferred.

The active compound can be administered to a mammalian host in a variety of forms, i.e., they may be combined with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, elixirs, syrups, injectable or eye drop solutions, and the like depending on the chosen route of administration, e.g., orally or parenterally. Parenteral administration in this respect includes administration by the following routes: intravenous, intramuscular, subcutaneous, intraocular, intrasynovial, transepithelial (including transdermal, ophthalmic, sublingual and buccal), topical (including ophthalmic, dermal, ocular, rectal, nasal inhalation via insufflation and aerosol), and rectal systemic.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, it may be enclosed in hard or soft shell gelatin capsules, compressed into tablets, or incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2% to about 6% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 1 and 1000 mg of active compound.

Tablets, troches, pills, capsules and the like may also contain the following: a binder such as polyvinylpyrrolidone, gum tragacanth, acacia, sucrose, corn starch or gelatin; an excipient such as calcium phosphate, sodium citrate and calcium carbonate; a disintegrating agent such as corn starch, potato starch, tapioca starch, 5 certain complex silicates, alginic acid and the like; a lubricant such as sodium lauryl sulfate, talc and magnesium stearate; a sweetening agent such as sucrose, lactose or saccharin; or a flavoring agent such as peppermint, oil of wintergreen or cherry flavoring. Solid compositions of a similar type are also employed as fillers in soft and hard-filled gelatin capsules; preferred materials in this connection also include lactose 10 or milk sugar as well as high molecular weight polyethylene glycols. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the 15 active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye, flavoring such as cherry or orange flavor, emulsifying agents and/or suspending agents, as well as such diluents as water, ethanol, propylene glycol, glycerin and various combinations thereof. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in 20 the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

The active compound may also be administered parenterally or intraperitoneally. For purposes of parenteral administration, solutions in sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous 25 solutions of the corresponding water-soluble, alkali metal or alkaline-earth metal salts previously enumerated. Such aqueous solutions should be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. Solutions of the active compound as a free base or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as 30 hydroxypropylcellulose. A dispersion can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. In

this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying technique which yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

For purposes of topical administration, dilute sterile, aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the above parenteral solutions, are prepared in containers suitable for drop-wise administration to the eye. The compounds of this invention may be administered to a mammal alone or in combination with pharmaceutically acceptable carriers. As noted above, the relative proportions of active ingredient and carrier are determined by the solubility and

chemical nature of the compound, chosen route of administration, the particular compound chosen and the physiological characteristics of the particular patient under treatment.

5 **KITS**

Yet another aspect of the invention is a kit for use in carrying out any of the above methods. A preferred embodiment is a kit comprising a GBS toxin receptor or fragment thereof. Preferably, the receptor or fragment is immobilized. A preferred kit can be used for identifying a compound that binds to GBS toxin receptor, and
10 comprises at least one cell that expresses GBS toxin receptor.

Another embodiment is a kit for monitoring tumor growth or metastasis, comprising a reagent for detecting expression of a GBS toxin receptor. Examples of such reagents include, but are not limited to, polynucleotide probes that hybridize to a GBS toxin receptor nucleic acid sequence and compounds that bind to a GBS toxin
15 receptor, such as, for example, an antibody that specifically recognizes GBS toxin receptor, a GBS toxin, a GBS toxin mimetic, or other compounds identified by the screening methods described above.

A third embodiment is a kit for purifying a compound that binds a GBS toxin receptor, comprising a GBS toxin receptor or fragment thereof that binds the compound. Preferred compounds include GBS toxin, GBS toxin mimetics, antibodies that specifically bind GBS toxin receptor, and other compounds identified by the screening methods described above.

Additional kit components can include, but are not limited to, additional reagents required for detection, a reference standard(s), instructions for use, and the like. Suitable reference standards include positive controls, negative controls, photographs of such controls, tabulated or graphed data of such controls, and the like. The kits may further comprise instructions for carrying out the methods described above, preferably printed instructions.

EXAMPLES

EXAMPLE 1 - CLONING SHEEP GBS TOXIN RECEPTOR

Primary culture of sheep lung endothelial cells

Small pieces of primary lung tissues from a 7-week old sheep are cut into small pieces in Hank's balanced salt solution (HBSS) containing 10 mM HEPES buffer (Life Technology), 1% penicillin/streptomycin and 0.1% gentamycin, and are cultured in sheep lung complete medium (Life Technology) at 37°C. After one week of the culture, clones of sheep lung endothelial cells are identified by Cobblestone morphology and harvested into 24-well tissue culture plates (Falcon) using cloning rings. When the cells are confluent, they are detached by pancreatin and transferred to a 60-mm tissue culture Petri dish or a T-25 tissue culture flask (Falcon). When they are confluent again, they are split and cultured into a few 100-mm tissue culture plates (Falcon). Each split is considered to be one passage. The same procedure is repeated until enough cells ($\sim 10^8$) are obtained for isolation of mRNA.

Isolation of mRNA and construction of cDNA library

Poly(A)⁺ RNA is isolated from 9.2×10^7 sheep lung endothelial cells (passage 8 and 9) by a standard method (Pharmacia). A total of 16 μg poly(A)⁺ RNA is acceptable amount obtained. 2.5 μg mRNA can be used to construct a cDNA library. Poly(A)⁺ RNA is oligo(dT)-primed (with *Not* I restriction site) and converted into double-stranded cDNA. After adding a *Bst*XI/*Eco*R I adaptor, the cDNA is unidirectionally cloned into the *Bst*XI and *Not* I sites of pCDNA3.1(+) (Invitrogen).

E. coli Top10F' (Invitrogen) is used as a host strain for amplification. 5.38×10^6 primary clones are an acceptable number generated. The library is amplified by plating cells onto fifty large LB agar plates containing ampicillin (100 µg/ml). The plates are scraped and aliquoted so that each aliquot represents 10 plates. DNA is purified by Qiagen Max columns (Qiagen).

Screening of cDNA library for a gene encoding GBS toxin receptor

To screen a cDNA library for a gene encoding GBS toxin receptor gene, a unique colorimetric method is used. Five µg plasmid DNA from each pool of cDNA library is used to transfect COS7 cells. The transfected cells are cultured in four to eight 96-well tissue culture plates (Falcon) for transient expression. Each well contains about 20,000 transfected cells in DMEM medium (Life Technology). COS7 cells transfected with pCDNA3.1(+) are used as a control. After 3 days expression, the medium is carefully removed. Each well is rinsed 3 times with HPSS buffer containing Mg^{2+} and Ca^{2+} (wash buffer) (Life Technology).

The cells are then incubated with biotinylated toxin (50 µl per well; 1 to 1.5 µg/ml) at room temperature for 1 h. After the hour incubation, the biotinylated toxin is discarded and the wells are rinsed 3 times with the wash buffer. The cells are incubated with streptavidin-β-gal solution and each well is rinsed 3 times with the wash buffer. The cells are then incubated with PNPG (50 µl per well; 1 mg/ml in substrate buffer) at 37°C. Absorbance at 405 nm is measured by an ELISA reader at 1 and 20 h, respectively. The cells which give the highest OD are harvested. Plasmid DNA is isolated by Hirt extraction. Plasmid DNA is amplified in *E. coli* to have enough DNA for the next transfection (enrichment).

Enrichment is done 8 times by this colorimetric method. The number of the transfected cells loaded into each well is gradually decreased in the last few enrichments and untransfected cells are added to each well to give a total number of 20,000 cells per well for the cells to be confluent and to reduce background after 3 days' expression. At the last enrichment, each well has only 1 to 10 transfected cells. Cells giving the highest OD are harvested. DNA is isolated and amplified in *E. coli*.

A number of isolated clones are individually assayed by this colorimetric method. The clones which showed higher binding to CM101 are sequenced.

Sequence analysis

DNA sequence analysis of clone pFU102, which has a 2.1kb insert, revealed a sequence encoding a partial integral glycoprotein. N-terminal sequence was obtained by 5'RACE method (Life Technology) and a full-length gene is designated as SP55. Triple ligation yielded pCD55, which contains an entire coding
5 region of SP55.

mRNA for the SP55 has 2844 nucleotides, encoding a protein of 495 amino acids with a predicated mass of 55 KDa, SP55. Analysis by the method of Klein et al. (Klein et al., *Biochim Biophys Acta*, 815:468-476 (1985)) classifies SP55 as an integral protein with seven transmembrane segments. SP55 has both N-glycosylation
10 and kinase phosphorylation sites. A Swiss-Prot. search of SP55 did not reveal any high homology to known human proteins. However, SP55 has some identity (~ 30%) to renal sodium-dependent phosphate transporters from human, rabbit, mouse and rat. In addition, SP55 has some identity (~ 30 to 39%) to hypothetical proteins (HYP50 and HYP63) from *C. elegans*.

15

EXAMPLE 2 - CLONING HUMAN GBS TOXIN RECEPTOR

The sheep GBS toxin receptor sequence shares about 37% identity with HYP50 and about 33% identity to HYP63, two hypothetical proteins from *C. elegans*. In the regions corresponding to amino acid residues 180-186 and 443-449 of
20 SEQ ID No. 2, five amino acids within a seven amino acid stretch are absolutely conserved among the three proteins.

A first degenerate oligonucleotide, CMR3-S: 5'-CGGGATCCCGCCNGCNATGCAYRSHRTSTGG-3' (SEQ ID No. 5), was designed to include all possible codons encoding the amino acid sequences of SP55, HYP50,
25 and HYP63 in the 180-186 region. A second degenerate oligonucleotide, CMR4-AS2: 5'-GGAATTCDDGGDGCRATKTCNARRTRRTT-3' (SEQ ID No. 6), was designed to include the complementary sequences of all possible codons encoding the amino acid sequences of SP55, HYP50, and HYP63 in the 443-449 region.

Polymerase chain reaction (PCR) was conducted using these
30 oligonucleotides and a human embryo lung cDNA library as a template. The reaction yielded three overlapping sequences approximately 400 bp in size, which encompass part of the nucleic acid sequence of SEQ ID No. 3. These sequences were then used as probes to clone the remainder of the gene, referred to herein and HP59 (SEQ ID NO: 7).

EXAMPLE 3 - PREPARATION OF ANTIBODIES AGAINST GBS TOXIN RECEPTOR

Rabbits are immunized with the synthetic peptides shown in Table 8. A 1-mg/ml solution of peptide plus KLH in 0.01M phosphate buffer is prepared. For the first immunization, 200 µg of peptide plus KLH (200 µl) and an equal volume of Freund's complete adjuvant, emulsified well before injection, is injected into 3-4 spots on the dorsal surface about the neck and shoulders of a rabbit. After two weeks, the second immunization (boost) is given at the same concentration of immunogen, but emulsified in Freund's incomplete adjuvant. The boost is delivered in the same region of the body. After another two weeks, blood is collected and assayed by ELISA for response against the peptide without KLH. Further boosts are given to improve antibody titer, if necessary.

Table 8**Immunogenic Peptides**

<u>Peptide</u>	<u>Amino Acid Sequence</u>	<u>Size</u>	<u>SEQ ID Ref.</u>
p56a	APSDGEEGSDRTPLLQRAPRAEPAPVC	27 aa	residues 8-35 of SEQ ID NO:4
p55a	LAPSDGEEGSDRTPL	15 aa	residues 7-22 of SEQ ID NO: 4
p57a	NTTAKDNRTSYECA	14 aa	residues 71-84 of SEQ ID NO: 4

Peptide p55 is a fragment of an extracellular domain of GBS toxin receptor. Peptide p57a is a fragment of an intracellular domain of GBS toxin receptor. Animals immunized with these peptides produce polyclonal antibodies Pab55 and Pab57, respectively.

EXAMPLE 4 - DETECTION OF GBS TOXIN RECEPTOR EXPRESSION IN TUMOR CELLS

This example shows that GBS toxin receptor can be detected in tumor cells. Immunohistochemistry is performed on paired human and mouse tissues of normal or tumor origin, using rabbit polyclonal antibodies Pab 55 and Pab 57.

Mouse and human tumor tissues are fixed in 10% neutral formalin. The tissues are then dehydrated, paraffin embedded and 10-20 x 8-micron sections are cut for immunohistochemical staining.

- Immunohistochemical analysis is performed with the automated Ventana Immunohistochemical Stainer according to the manufacturer's suggested protocol (Ventana, Tucson, Arizona). Sections are deparaffinated with xylene. The prepared sections are then treated with 1% hydrogen peroxide prepared in 30% aqueous methanol for 20 minutes at room temperature to quench endogenous peroxidase activity. The slides are then washed with PBS, blocked with 5% BSA and 5% goat serum in PBS, washed again and then incubated for 30 minutes at 37°C with the appropriate diluted (1:100) antibody. Horseradish peroxidase-labeled goat anti-rabbit IgG is used as a secondary antibody. For visualization, the sections are incubated with DAB/H₂O₂. The sections are finally incubated with a copper enhancer (Ventana) for 4 minutes, washed, counterstained with hematoxylin, and mounted in toluene-minus mounting medium. Photographic documentation is performed and images are stored for later review and analysis. The results are summarized in Table 9. The numbers refer to glass slides.

TABLE 9

20 **Immunohistochemistry of tumor and normal tissues**
(diff. = differentiated)

Human tissues:

	<u>Antibody</u>	<u>Magnification</u>	<u>Signal</u>
1. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 55	400x	+
2. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 55	400x	+
3. Normal ovary (96-08ZO08) control tissue	Pab 55	400x	-
4. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 57	400x	+
5. Ovary tumor (9 5 -02VO 16) high grade papillary carcinoma	Pab 57	400x	+
6. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 57	400x	+
7. Normal ovary 96-08ZO08) control	Pab 57	400x	-
8. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 55	400x	+
9. Normal colon 9708VO08) control	Pab 55	400x	-
10. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 57	400x	+

11. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 57	400x	+
12. Normal colon 9708VO08) control	Pab 57	400x	-
13. Female breast cancer (97-IOV03a) Invasive mammary carcinoma	Pab 55	400x	+
14. Male breast cancer (no code) mammary carcinoma	Pab 55	400x	+
15. Normal female breast 97-12VO20-3) control	Pab 55	400x	-
16. Female breast cancer 97-IOV03a) Invasive mammary carcinoma	Pab 57	400x	+
17. Male breast cancer (no code) mammary carcinoma	Pab 57	400x	+
18. Normal female breast (97-12VO20-3) control	Pab 57	400x	-
19. Lung cancer (97- 1 OV022-5) poorly diff. NOJ-small cell carcinoma	Pab 55	400x	+
20. Normal lung (98-0 1 VO 11) control	Pab 55		-
21. Lung cancer (97-10VO22-5) poorly diff. NOJ-small cell carcinoma	Pab 57	400x	+
22. Lung cancer (97-10VO22-5) poorly diff. NOJ-small cell carcinoma	Pab 57	400x	+
23. Normal lung (98-0 1 VO 11) control	Pab 57		-

Mouse Tissues:

	<u>Antibody</u>	<u>Magnification</u>	<u>Signal</u>
24. Madison Lung Tumor (MLT) untreated with CM 101	Pab 55		+
25. MLT untreated with CM 101	Pab 55		+
26. Normal mouse lung	Pab 55		-
27. MLT untreated with CM 101	Pab 57		+
28. Normal mouse lung	Pab 57		-

The Pab 55 antibody stains the cells lining a blood vessel in a human ovary cancer tissue section, but such staining is not apparent in cells of normal human ovary tissue (see FIG. 2A and 2B, respectively). Similar results are obtained with the Pab 57 antibody (see FIG. 3A and 3B). As shown in the above table and in FIGS. 2A-3B, antibodies raised to GBS toxin receptor fragments specifically bound to tumor tissues but not normal tissues, suggesting that GBS toxin receptor is expressed in tumor cells but not normal cells.

10 **EXAMPLE 5— DETECTION OF GBS TOXIN RECEPTOR EXPRESSION IN MICE AFFLICTED WITH RHEUMATOID ARTHRITIS**

This example shows that GBS toxin receptor can be detected in cells from a mammalian model for rheumatoid arthritis (RA). Mice with collagen-induced arthritis were treated with CM101 or carrier. CM101 reversed the inflammatory

damage and inhibited pannus formation. Mouse #8 and #15, which were treated with CM101, and two control mice (not treated with CM101) were sacrificed for immunohistochemistry.

5

TABLE 10
Immunohistochemistry of Rheumatoid Arthritic Mice

29. No CM 101	Pab 55	+
30. MOUSE 8 - 5' (vessel)	Pab 55	+
31. No CM 101	Pab 57	+
32. MOUSE 15 - 5' (vessel)	Pab 57	+
33. MOUSE 8 - 5' (between joint)	Pab 57	+
34. MOUSE 15 - 5'	Pab 57	+
35. No CM 101 (marrow)	Pab 57	+
36. MOUSE 15 - 5' (marrow)	Pab 57	+

As shown above Pab55 and Pab57 specifically bound to pathologic neovasculature in the pannus, suggesting that GBS toxin receptor is expressed in mice afflicted with rheumatoid arthritis. No binding of CM101 was observed in the normal neovasculature in the growth plate of the joints of the arthritic mice.

EXAMPLE 6 – TARGETED DELIVERY OF A CHIMERIC COMPOUND TO TISSUES EXPRESSING GBS TOXIN RECEPTOR

This example shows the targeted delivery of a chimeric compound to tissues expressing GBS toxin. The chimeric compound is a CM101-biotin conjugate. Mice with Madison Lung Tumors (MLT) are infused intravenously (i.v.) with biotinylated CM 101.

CM101 has been reacted with hydrazinylated biotin to form the biotin hydrazone at the reducing end of the polysaccharide CM101. Briefly, 25 micrograms of lyophilized CM101 is dissolved in 250 μ l labeling buffer at 100 mM sodium acetate, 0.02% sodium azide. Aqueous meta-periodate (125 μ l of 30 mM) is added and the oxidation is allowed to proceed in the dark for 30 minutes at room temperature. The reaction is terminated by adding 80 mM Na_2SO_3 to the solution. The resultant aldehydes are reacted with 125 μ l of 5 mM NHS-LC-Biotin (MW 556.58) for a 1 hour incubation at room temperature to form biotinylated CM101. Excess biotin is removed by dialysis against 1 liter of PBS at 4°C four times. The

product is purified by gel filtration on an Ultrahydrogel 1000 HPLC. lyophilized and stored at -70°C until use.

Tissues are recovered 5 min post infusion with CM101 and subjected to immunohistochemistry. Tumor and normal mouse tissue sections are analyzed for CM
5 101 binding by both mouse anti-CM101 mAb (7A3), followed by secondary mAb-
HRP conjugate (referred to in FIG. 4B as MLT CM101-Biot.5' + McAb), or with
avidin (which specifically binds biotin) conjugated with HRP (referred to in FIG. 4A
as MLT CM101-Biot.5' + Strep.HRP).

FIGS. 4A-4C depict different sections taken from the same tumor and include
10 a longitudinal view of the same blood vessel approximately in the center of the
figures. The dark staining in FIG. 4A shows the localization of the biotin component
in the cells lining the blood vessel. Similarly, FIG. 4B depicts the localization of the
CM101 component in the cells lining the blood vessel. FIG. 4C is a negative control
that was not exposed to CM101. The analysis clearly shows that 7A3 and avidin bind
15 to the same blood vessels in tumor tissue. Thus, biotin has been delivered to the
blood vessel of the tumor tissue by virtue of its physical association with a compound
(CM101) that binds the GBS toxin receptor.

These studies show that chimeric compounds can be delivered to tissues
undergoing pathologic and/or hypoxia-driven angiogenesis or neovascularization. As
20 part of a chimeric compound, cytotoxic molecules can be directed to such tissues, e.g.,
tumor tissue. The cytotoxic molecule can be coupled directly to a molecule that binds
GBS toxin receptor, e.g., GBS toxin. Alternatively, the molecule that binds GBS
toxin receptor can be coupled to biotin and the cytotoxic molecule can be coupled to
avidin.

EXAMPLE 7 – ENHANCED SENSITIVITY TO GBS-TOXIN-DEPENDENT CYTOTOXICITY OF CELLS EXPRESSING GBS TOXIN RECEPTOR

This example shows the enhanced sensitivity to GBS-toxin-dependent cytotoxicity of cells transfected with the GBS toxin receptor, relative to control cells.

- 5 Without being bound to a particular theory, the inventors believe that complement binds GBS toxin bound to the GBS toxin receptor on a cell, thereby targeting the cell for killing by white blood cells (WBC).

Human bladder carcinoma cells (ECV cells), are stable transfected with the human GBS toxin receptor gene. The resultant cell line is ECV711. Cells stable
10 transfected with vector alone as referred to as V23. ECV 711 and V23 are seeded in 96-well plates at 5,000 cells/well.

White blood cells are collected from healthy human donors as follows. Blood is collected by standard phlebotomy procedures into heparinized tubes (30 U/ml) and centrifuged at 2000 rpm for 20 min. The interface is carefully transferred to a new
15 tube and washed twice by centrifugation with medium (RPMI-1640). Cells are resuspended in RPMI-1640 supplemented with 5% fetal bovine serum (FBS) and Interferon-gamma (IFN) at 100 U/ml, and incubated overnight in a 37°C, 5%CO₂ incubator. The cells are then resuspended in fresh medium with 5% FBS.

5,000 cells of the WBC preparation are added to each well containing the
20 transfected cells. CM101 is added to a final concentration of 1µg/ml to the wells together with human serum from matching human donors. The cells are incubated 6 hours at 37 ° C.

Cytotoxicity is assayed by measuring lactate dehydrogenase (LDH) using the Promega's CytoTox 96 Non-Radioactive Assay kit (Nachlas et al. (1960) *Anal. Biochem* 1, 317; Korzeniewski et al. (1983) *J. Immunol. Methods* 64, 313; Decker et al. *J. Immunol. Methods* 115, 61; Brander et al. (1993) *Eur. J. Immunology* 23, 3217; Behl et al. (1994) *Cell* 77, 817; Lappalainen et al. (1994) *Pharm. Research* 11, 1127; Allen et al. (1994) *Promega Notes* 45, 7; Sinensky et al. (1995) *Toxicol. Letters* 75, 02; Moravec (1994) *Promega Notes* 45, 11). Percent cytotoxicity is calculated as
30 recommended by the manufacturer's instructions. The results are shown in Table 11.

Table 11

Cytotoxicity	ECV 711	V 23
WBC, IFN, C3, -CM101	29.1%	27.5%
WBC, IFN, C3, +CM101	40.45%	22.46%

There is an increase in cytotoxicity of 39% when the ECV 711 cells are incubated with CM101, WBC and human serum (source of C3) compared to cells
5 incubated without CM101. Control cells transfected with vector alone, V23, do not show a CM101 dependent increase in cytotoxicity.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by
10 reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide at least 10 bases in length comprising a nucleic acid sequence encoding, or complementary to a nucleic acid sequence encoding, a mammalian receptor for group B β -hemolytic Streptococcus toxin (GBS toxin receptor), or a polypeptide fragment thereof.
5
2. The polynucleotide of Claim 1, wherein the nucleic acid sequence comprises SEQ ID NO: 9.
3. The polynucleotide of Claim 1, wherein the nucleic acid sequence has 100% identity to a nucleic acid sequence selected from the group consisting of
10 residues 61 to 1542 of SEQ ID NO: 1, residues 266 to 1870 of SEQ ID NO: 7, and residues 87 to 1568 of SEQ ID NO: 3.
4. The polynucleotide of Claim 1, wherein the polynucleotide is hybridizable under high stringency conditions to the nucleic acid sequence of SEQ ID NO: 7.
5. A vector comprising the polynucleotide of Claim 1.
- 15 6. A host cell transformed with the vector of Claim 5.
7. A process for producing a mammalian GBS toxin receptor or fragment thereof, comprising culturing the host cell of Claim 6 in a suitable culture medium.
8. An isolated polypeptide comprising a mammalian GBS toxin receptor or fragment thereof.
- 20 9. The polypeptide of Claim 8, wherein the receptor has at least about 86% identity to the corresponding amino acid sequence of SEQ ID NO: 2.
10. The polypeptide of Claim 8, wherein the receptor or fragment has 100% identity to the corresponding region of the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 8.
- 25 11. The polypeptide of Claim 8, wherein the polypeptide is encoded by a nucleic acid sequence hybridizable under high stringency conditions to a nucleic acid sequence selected from the group consisting of:

- a) nucleotides 61 to 1542 of SEQ ID NO: 1, and
- b) nucleotides 87 to 1568 of SEQ ID NO: 3.

12. An isolated polypeptide comprising an amino acid sequence that differs from an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:8 at no more than about 20% of the amino acid residues.

13. The isolated polypeptide of Claim 12, wherein the amino acid sequence of said isolated polypeptide differs from the amino acid sequence selected from said group by one amino acid residue.

14. The isolated polypeptide of Claim 12, wherein the different amino acid residues are conservative substitutions of the corresponding residues of the amino acid sequence selected from said group.

15. An isolated polypeptide comprising an amino acid sequence of the formula:

AA1-AA_n-AA_m

wherein:

AA1 is absent or is M;

AA_n is a contiguous chain of 0 to 100 amino acids, preferably of 0 or 41 amino acids, even more preferably of residues 2-42 of SEQ ID NO:8; and

AA_m is a contiguous chain of 494 amino acids comprising AA43 through AA536, wherein:

(1) each of AA43, AA47, AA51, AA52, AA57, AA58, AA65, AA66, AA72, AA85, AA87, AA93, AA94, AA96, AA115, AA116, AA122, AA123, AA125, AA134, AA143, AA173, AA174, AA178, AA185, AA186, AA189, AA190, AA196, AA200, AA204, AA206, AA207, AA220, AA253, AA260, AA276, AA277, AA280, AA283, AA287, AA294, AA295, AA298, AA300, AA301, AA312, AA324, AA326, AA360, AA365, AA373, AA374, AA379, AA396, AA403, AA407, AA418, AA480, AA483, AA486, AA491, AA494, AA502, AA528, AA529, AA532 and AA536 is an amino acid residue corresponding to:

(a) residue 43, 47, 51, 52, 57, 58, 65, 66, 72, 85, 87, 93, 94, 96, 115, 116, 122, 123, 125, 134, 143, 173, 174, 178, 185, 186, 189, 190, 196, 200, 204, 206, 207, 220, 253, 260, 276, 277, 280, 283, 287, 294, 295, 298, 300, 301, 312, 324, 326, 360, 365, 373, 374, 379, 396, 403, 407, 418, 480, 483, 486, 491, 494, 502, 528, 529, 532 and 536, respectively, of SEQ ID NO:8;

(b) residue 2, 6, 10, 11, 16, 17, 24, 25, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283,

285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495, respectively of SEQ ID NO:4; or

(c) a conservative substitution thereof;

- (2) each of AA44-AA46, AA48-AA50, AA53-AA56, AA59-AA64, AA67-AA71, AA73-AA84, AA86, AA88-AA92, AA95, AA97-AA114, AA117-AA121, AA124, AA126-AA133, AA135-AA142, AA144-AA172, AA175-AA177, AA179-AA184, AA187-AA188, AA191-AA195, AA197-AA199, AA201-AA203, AA205, AA208-AA219, AA221-AA252, AA254-AA259, AA261-AA275, AA278-AA279, AA281-AA282, AA284-AA286, AA288-AA293, AA296-AA297, AA299, AA302-AA311, AA313-AA323, AA325, AA327-AA359, AA361-AA364, AA366-AA372, AA375-AA378, AA380-AA395, AA397-AA402, AA404-AA406, AA408-AA417, AA419-AA478, AA481-AA482, AA484-AA485, AA487-AA490, AA492-AA493, AA495-AA501, AA503-AA527, AA530-AA531 and AA533-AA535 is
- (a) residue 44-46, 48-50, 53-56, 59-64, 67-71, 73-84, 86, 88-92, 95, 97-114, 117-121, 124, 126-133, 135-142, 144-172, 175-177, 179-184, 187-188, 191-195, 197-199, 201-203, 205, 208-219, 221-252, 254-259, 261-275, 278-279, 281-282, 284-286, 288-293, 296-297, 299, 302-311, 313-323, 325, 327-359, 361-364, 366-372, 375-378, 380-395, 397-402, 404-406, 408-417, 419-478, 481-482, 484-485, 487-490, 492-493, 495-501, 503-527, 530-531 and 533-535, respectively, of SEQ ID NO:8; or

(b) a conservative substitutions thereof; and

(3) one or more of AA315 through AA367 are optionally absent.

16. An antibody that recognizes a mammalian GBS toxin receptor or fragment thereof.

17. An isolated complex comprising a GBS toxin bound to a mammalian GBS toxin receptor or fragment thereof.

18. A method of forming a complex comprising:

contacting a GBS toxin with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide, and allowing the complex to form.

19. A method for purifying a compound that binds a GBS toxin receptor, which method comprises:

providing a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that binds GBS toxin; contacting said polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide; and

separating the bound compound from the remainder of the sample.

20. A method of determining the presence or absence of GBS toxin in a sample, which method comprises:

5 contacting the sample with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide,
and determining whether specific binding has occurred.

21. A method for diagnosing early onset disease in a neonate comprising performing the method of Claim 20, wherein the sample is obtained from the neonate
10 and wherein presence of the GBS toxin is indicative of early onset disease.

22. A method for detecting pathologic vasculature in a mammalian tissue, which method comprises detecting the presence of a GBS toxin receptor.

23. A method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor, comprising:

15 combining a test compound with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, in a reaction mixture containing GBS toxin and under conditions that permit specific binding of the GBS toxin to the receptor or fragment, and
determining the amount of inhibition by the compound of the binding
20 of the GBS toxin to the polypeptide.

24. An inhibitor of binding of a GBS toxin to a mammalian GBS toxin receptor.

25. A method for identifying a compound which specifically binds a mammalian GBS toxin receptor, comprising:

25 combining a test compound with a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that can bind GBS toxin, under conditions that allow specific binding to occur, and
detecting a complex formed between said test compound and said

polypeptide.

26. A method for determining cytotoxicity of a test chimeric compound, which method comprises:

- 5 exposing a cell expressing, on the cell surface, a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, to a test chimeric compound comprising a cytotoxic agent coupled to said GBS toxin; and
detecting signs of cytotoxicity.

27. A chimeric compound comprising a cytotoxic agent covalently linked to a molecule that specifically binds a mammalian GBS toxin receptor.

- 10 28. A method for identifying an inhibitor of GBS toxin receptor, which method comprises:

- incubating test cells in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, wherein the test cells express GBS toxin receptor or a
15 fragment thereof having GBS toxin receptor activity; and
comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being an
20 inhibitor of the GBS toxin receptor.

29. A method for identifying an inhibitor of endothelial cell proliferation or migration, which method comprises:

- incubating test endothelial cells in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test
25 compound can proliferate or migrate, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity; and
comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration

in the presence of the test compound is indicative of the test compound being an inhibitor of the endothelial cell proliferation or migration.

30. A method for identifying a therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic angiogenesis or neovascularization, which method comprises:

incubating test cells in the presence and absence of a test compound, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity;

- comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being a candidate therapeutic compound for the treatment or prevention of the medical condition.

31. The method of Claim 30, wherein the medical condition is a cancerous tumor.

32. The method of Claim 30, wherein the medical condition is a reperfusion injury.

33. The method of Claim 30, wherein the medical condition is scarring during wound healing.

34. The method of Claim 30, wherein the medical condition is keloids.

35. The method of Claim 30, wherein the medical condition is a chronic inflammatory disease.

36. The method of Claim 30, wherein the medical condition is neural injury.

37. A method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor, comprising:

(a) simulating and selecting the most probable conformations of a mammalian GBS toxin receptor,

- (b) designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the polypeptide,
- (c) chemically synthesizing the analog, and
- (d) evaluating the bioactivity of the analog.

5 38. A method for identifying a compound which binds to a mammalian GBS toxin receptor, comprising:

- (a) simulating and selecting the most probable conformations of a mammalian GBS toxin receptor,
- (b) deducing the most probable binding domains of the polypeptide,
- 10 (c) designing a compound that would form the energetically most probable complexes with the polypeptide,
- (d) chemically synthesizing the compound, and
- (e) evaluating the bioactivity of the compound.

15 39. A method for the prevention or treatment of neonatal onset disease in a human neonate, comprising administering an inhibitor of binding of GBS toxin to a human GBS toxin receptor.

20 40. A method for inhibiting pathologic or hypoxia-driven endothelial cell proliferation or migration in a mammalian tissue, which method comprises specifically binding a molecule to a GBS toxin receptor present on the surface of at least one cell in the tissue, the molecule being selected from the group consisting of:

- a compound that can evoke an inflammatory response when bound to a GBS toxin receptor in a mammal;
- a chimeric compound comprising a cytotoxic compound coupled to a compound that specifically binds the GBS toxin receptor;
- 25 an inhibitor of GBS toxin receptor phosphorylation; and
- an inhibitor of GBS toxin receptor activity.

41. A pharmaceutical composition comprising a pharmaceutically effective amount of a molecule selected from the group consisting of:

- a GBS toxin receptor or fragment thereof;

an inhibitor of a GBS toxin receptor; and
a chimeric compound comprising a cytotoxic agent coupled to a
compound that binds GBS toxin receptor,
and a pharmaceutically acceptable carrier.

5

42. A kit comprising a component selected from the group consisting of:

a GBS toxin receptor or fragment;

a reagent for detecting the presence of a GBS toxin receptor or
fragment; and

10

a reagent for detecting the presence of polynucleotide encoding the
GBS toxin receptor or fragment.

43. A molecule for use in a method of treatment of the human or animal body,
said molecule being selected from the group consisting of:

15 a GBS toxin receptor or fragment thereof for use in a method of
treatment of the human or animal body, said molecule being selected from the group
consisting of:

a GBS toxin receptor or fragment thereof;

an inhibitor of binding of GBS toxin to a GBS toxin receptor;

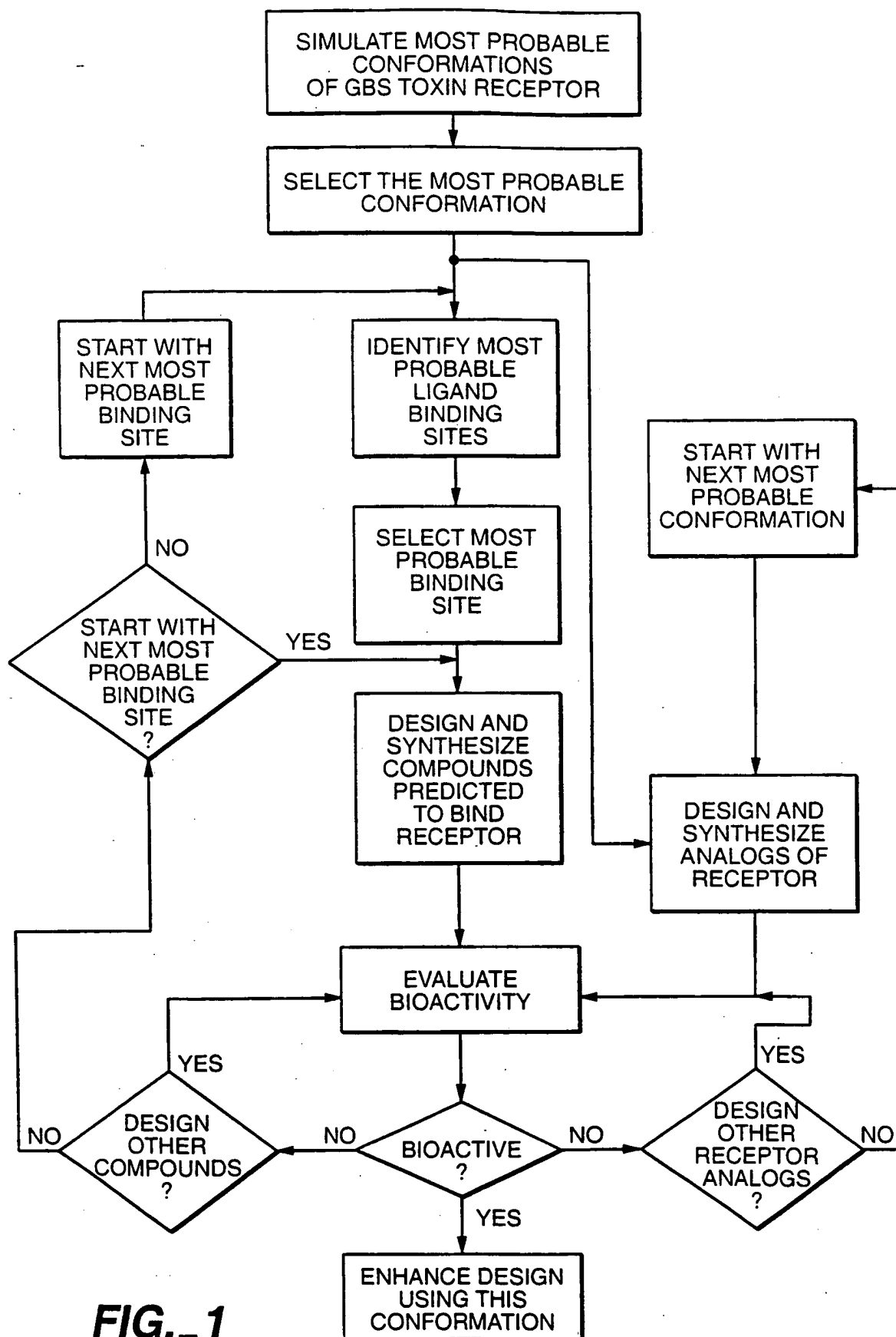
an inhibitor of a GBS toxin receptor; and

20

a chimeric compound comprising a cytotoxic agent coupled to a
compound that binds GBS toxin receptor.

44. Use of an inhibitor of a GBS toxin receptor, or of an inhibitor of binding of
GBS toxin to a GBS toxin receptor, for the manufacture of a medicament for the
treatment of a medical condition characterized by pathologic or hypoxia-driven
25 angiogenesis or neovascularization.

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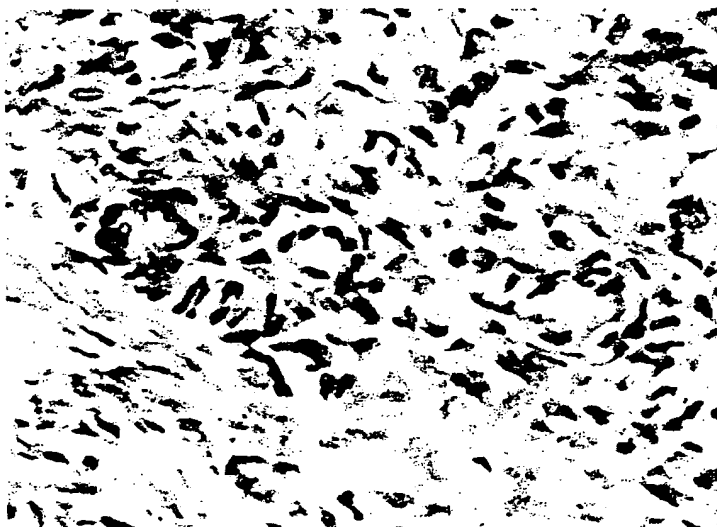
**FIG._1**

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HUMAN OVARY
CANCER + Pab 55

FIG. 2A



NORMAL HUMAN
OVARY + Pab 55

FIG. 2B

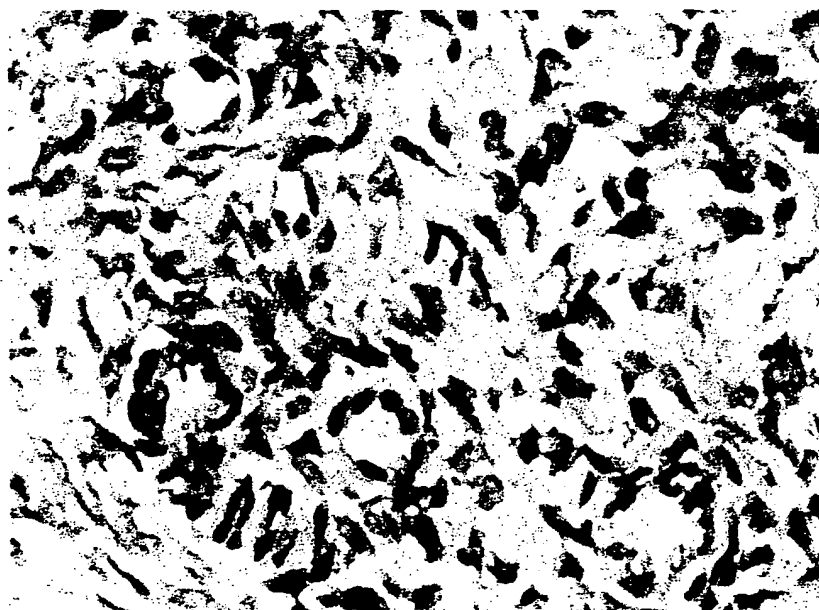
SUBSTITUTE SHEET (RULE 26)

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HUMAN OVARY
CANCER + Pab 57

FIG._3A



NORMAL HUMAN
OVARY + Pab 57

FIG._3B

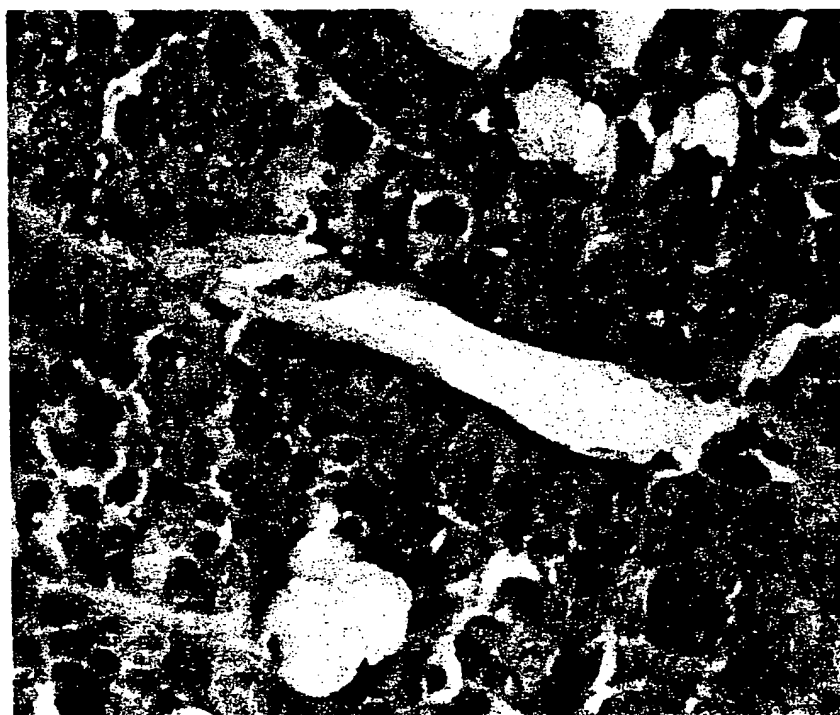
SUBSTITUTE SHEET (RULE 26)

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MLT CM101 - Biot.5' + Strep.HRP

FIG. 4A

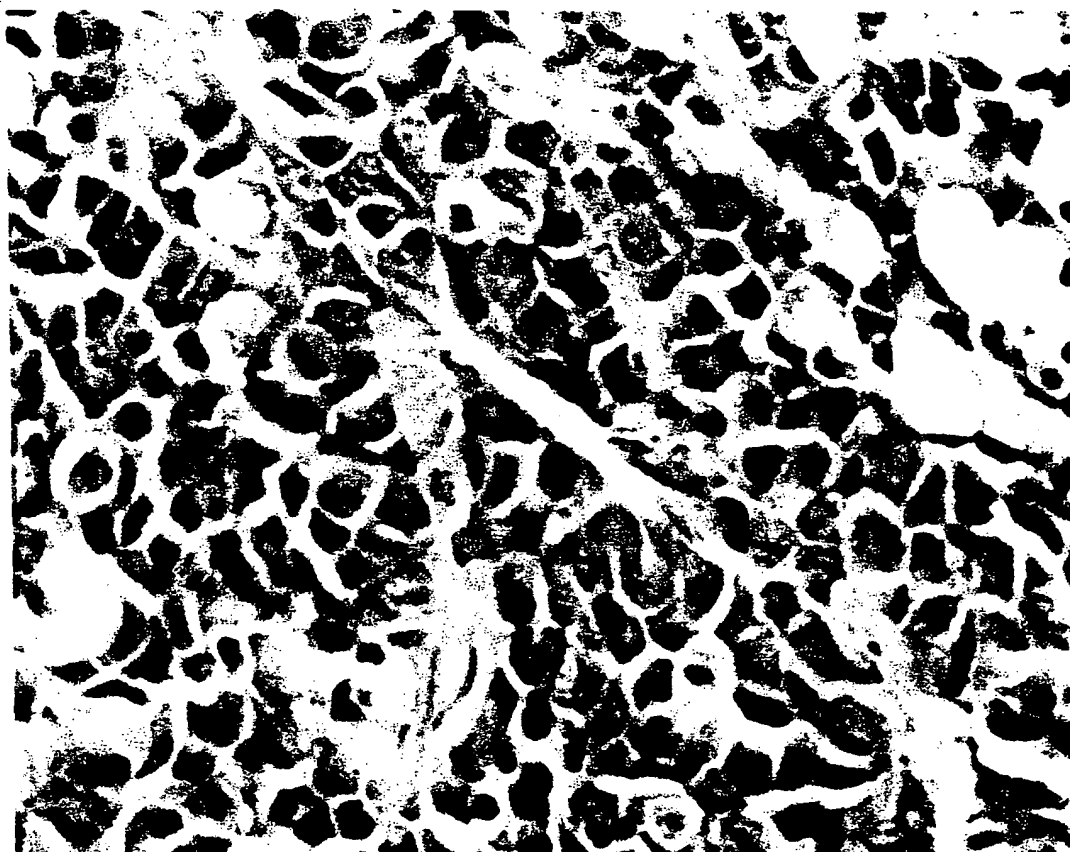


MLT CM101 - Biot.5' + mAb

FIG. 4B

SUBSTITUTE SHEET (RULE 26)

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MLT - PBS 5' + Streptavidin - HRP

FIG. 4C

SUBSTITUTE SHEET (RULE 26)

SEQUENCE LISTING

<110> Hellerqvist, Carl
Fu, Changlin

<120> GBS Toxin Receptor

<130> CARB-008/01WO

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 130 135 140
 Ala Thr Ala Ile Phe Thr Leu Phe Thr Pro Leu Ala Ala Asp Phe Gly
 145 150 155 160
 Val Gly Ala Leu Val Ala Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly
 165 170 175
 Val Thr Tyr Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro
 180 185 190
 Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu
 195 200 205
 Gly Thr Val Val Ser Leu Pro Leu Ser Gly Val Ile Cys Tyr Tyr Met
 210 215 220
 Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Ile Val Gly Ile Ile Trp
 225 230 235 240
 Phe Ile Leu Trp Ile Cys Leu Val Ser Asp Thr Pro Glu Thr His Lys
 245 250 255

Thr Ile Thr Pro Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Lys Asn
 260 265 270
 Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Ile Pro Met Leu Lys Ser
 275 280 285
 Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr
 290 295 300
 Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Val Leu
 305 310 315 320
 Arg Phe Asn Ile Gln Glu Asn Gly Phe Leu Ser Ala Val Pro Tyr Leu
 325 330 335
 Gly Cys Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu
 340 345 350
 Arg Ala Arg Trp Asn Phe Ser Thr Leu Trp Val Arg Arg Val Phe Ser
 355 360 365
 Leu Ile Gly Met Ile Gly Pro Ala Ile Phe Leu Val Ala Ala Gly Phe
 370 375 380
 Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr
 385 390 395 400
 Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp
 405 410 415
 Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe
 420 425 430
 Ala Thr Ile Pro Gly Met Ile Gly Pro Ile Ile Ala Arg Ser Leu Thr
 435 440 445
 Pro Glu Asn Thr Ile Gly Glu Trp Gln Thr Val Phe Cys Ile Ala Ala
 450 455 460
 Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
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 485 490 495

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<211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: PCR primer

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31

<210> 6
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: PCR primer

<400> 6
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29

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 <211> 2930
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (263)..(1870)

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 gcgggagacg gtcgtccgaa caccggctcc ccggcatgcg tagaccggcg ggcggagcgg 180
 gctcactttg cgccaatcct acgagaactc ccagaactcc gcttccttag tccaacccaa 240
 gccagagttg cccacaccta ag atg gcg gcg ggg gcg atg aca ccg ccc cgc 292
 Met Ala Ala Gly Ala Met Thr Pro Pro Arg
 1 5 10

ccg gtc cag cca gct cgg ccc ggg ggc ttc ggg ctg tcg ggc cgg cgc 340
 Pro Val Gln Pro Ala Arg Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg
 15 20 25

tcc ctt ctc tgc cag gtg gcg agt aca cct gct cac gta ggc gtc atg 388
 Ser Leu Leu Cys Gln Val Ala Ser Thr Pro Ala His Val Gly Val Met
 30 35 40

agg tct ccg gtt cga gac ctg gcc cgg aac gat ggc gag gag agc acg 436
 Arg Ser Pro Val Arg Asp Leu Ala Arg Asn Asp Gly Glu Glu Ser Thr
 45 50 55

gac cgc acg cct ctt cta ccg ggc gcc cca cgg gcc gaa gcc gct cca 484
 Asp Arg Thr Pro Leu Leu Pro Gly Ala Pro Arg Ala Glu Ala Ala Pro
 60 65 70

gtg tgc tgc tct gct cgt tac aac tta gca att ttg gcc ttt ttt ggt 532
 Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Ile Leu Ala Phe Phe Gly
 75 80 85 90

ttc ttc att gtg tat gca tta cgt gtg aat ctg agt gtt gcg tta gtg 580
 Phe Phe Ile Val Tyr Ala Leu Arg Val Asn Leu Ser Val Ala Leu Val
 95 100 105

gat atg gta gat tca aat aca act tta gaa gat aat aga act tcc aag 628
 Asp Met Val Asp Ser Asn Thr Thr Leu Glu Asp Asn Arg Thr Ser Lys
 110 115 120

gcg tgt cca gag cat tct gct ccc ata aaa gtt cat cat aat caa acg 676
 Ala Cys Pro Glu His Ser Ala Pro Ile Lys Val His His Asn Gln Thr
 125 130 135

ggt aag aag tac caa tgg gat gca gaa act caa gga tgg att ctc ggt 724
 Gly Lys Lys Tyr Gln Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu Gly
 140 145 150

tcc ttt ttt tat ggc tac atc atc aca cag att cct gga gga tat gtt 772
 Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr Val
 155 160 165 170

gcc agc aaa ata ggg ggg aaa atg ctg cta gga ttt ggg atc ctt ggc 820
 Ala Ser Lys Ile Gly Gly Lys Met Leu Leu Gly Phe Gly Ile Leu Gly
 175 180 185

act gct gtc ctc acc ctg ttc act ccc att gct gca gat tta gga gtt 868
 Thr Ala Val Leu Thr Leu Phe Thr Pro Ile Ala Ala Asp Leu Gly Val
 190 195 200

gga cca ctc att gta ctc aga gca cta gaa gga cta gga gag ggt gtt 916
 Gly Pro Leu Ile Val Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly Val
 205 210 215

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aca ttt cca gcc atg cat gcc atg tgg tct tct tgg gct ccc cct ctt 964
Thr Phe Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro Leu
220 225 230

gaa aga agc aaa ctt ctt agc att tcg tat gca gga gca cag ctt ggg 1012
Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu Gly
235 240 245 250

aca gta att tct ctt cct ctt tct gga ata att tgc tac tat atg aat 1060
Thr Val Ile Ser Leu Pro Leu Ser Gly Ile Ile Cys Tyr Tyr Met Asn
255 260 265

tgg act tat gtc ttc tac ttt ttt ggt act att gga ata ttt tgg ttt 1108
Trp Thr Tyr Val Phe Tyr Phe Phe Gly Thr Ile Gly Ile Phe Trp Phe
270 275 280

ctt ttg tgg atc tgg tta gtt agt gac aca cca caa aaa cac aag aga 1156
Leu Leu Trp Ile Trp Leu Val Ser Asp Thr Pro Gln Lys His Lys Arg
285 290 295

att tcc cat tat gaa aag gaa tac att ctt tca tca tta aga aat cag 1204
Ile Ser His Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Arg Asn Gln
300 305 310

ctt tct tca cag aag tca gtg ccg tgg gta ccc att tta aaa tcc ctg 1252
Leu Ser Ser Gln Lys Ser Val Pro Trp Val Pro Ile Leu Lys Ser Leu
315 320 325 330

cca ctt tgg gct atc gta gtt gca cac ttt tct tac aac tgg act ttt 1300
Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr Phe
335 340 345

tat act tta ttg aca tta ttg cct act tat atg aag gag atc cta agg 1348
Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Ile Leu Arg
350 355 360

ttc aat gtt caa gag aat ggg ttt tta tct tca ttg cct tat tta ggc 1396
Phe Asn Val Gln Glu Asn Gly Phe Leu Ser Ser Leu Pro Tyr Leu Gly
365 370 375

tct tgg tta tgt atg atc ctg tct ggt caa gct gct gac aat tta agg 1444
Ser Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu Arg
380 385 390

gca aaa tgg aat ttt tca act tta tgt gtt cgc aga att ttt agc ctt 1492
Ala Lys Trp Asn Phe Ser Thr Leu Cys Val Arg Arg Ile Phe Ser Leu
395 400 405 410

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 Ile Gly Met Ile Gly Pro Ala Val Phe Leu Val Ala Ala Gly Phe Ile
 415 420 425

ggc tgt gat tat tct ttg gcc gtt gct ttc cta act ata tca aca aca 1588
 Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr Thr
 430 435 440

ctg gga ggc ttt tgc tct tct gga ttt agc atc aac cat ctg gat att 1636
 Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp Ile
 445 450 455

gct cct tcg tat gct ggt atc ctc ctg ggc atc aca aat aca ttt gcc 1684
 Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe Ala
 460 465 470

act att cca gga atg gtt ggg ccc gtc att gct aaa agt ctg acc cct 1732
 Thr Ile Pro Gly Met Val Gly Pro Val Ile Ala Lys Ser Leu Thr Pro
 475 480 485 490

gat aac act gtt gga gaa tgg caa acc gtg ttc tat att gct gct gct 1780
 Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala Ala
 495 500 505

att aat gtt ttt ggt gcc att ttc ttt aca cta ttc gcc aaa ggt gaa 1828
 Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly Glu
 510 515 520

gta caa aac tgg gct ctc aat gat cac cat gga cac aga cac 1870
 Val Gln Asn Trp Ala Leu Asn Asp His His Gly His Arg His
 525 530 535

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 aaagtgcctt ctgtattgtg taagcattct atgtcttttt ttaattgtac ttgtattaga 1990
 tttttaaggc ctataatcat gaaatatcac tagttgccag aataataaaa tgaactgtgt 2050
 ttaattatga ataatatgta agctaggact tctacttttag gttcacatac ctgcctgcta 2110
 gtcggggcaac atgaagtagg acagttctgt tgattttttta gggccatact aaagggaatg 2170
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 aatattggct ggcattgcaac ctgcaaaaaga aaaatgcgaa gcggccgggc acggtgggtc 2650
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<210> 8

<211> 536

<212> PRT

<213> Homo sapiens

<400> 8

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			20					25					30		
Ala Ser Thr Pro Ala His Val Gly Val Met Arg Ser Pro Val Arg Asp															
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Leu Ala Arg Asn Asp Gly Glu Glu Ser Thr Asp Arg Thr Pro Leu Leu															
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Pro Gly Ala Pro Arg Ala Glu Ala Ala Pro Val Cys Cys Ser Ala Arg															
	65				70					75				80	
Tyr Asn Leu Ala Ile Leu Ala Phe Phe Gly Phe Phe Ile Val Tyr Ala															
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Leu Arg Val Asn Leu Ser Val Ala Leu Val Asp Met Val Asp Ser Asn															
			100					105						110	

Thr Thr Leu Glu Asp Asn Arg Thr Ser Lys Ala Cys Pro Glu His Ser
 115 120 125
 Ala Pro Ile Lys Val His His Asn Gln Thr Gly Lys Lys Tyr Gln Trp
 130 135 140
 Asp Ala Glu Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr
 145 150 155 160
 Ile Ile Thr Gln Ile Pro Gly Gly Tyr Val Ala Ser Lys Ile Gly Gly
 165 170 175
 Lys Met Leu Leu Gly Phe Gly Ile Leu Gly Thr Ala Val Leu Thr Leu
 180 185 190
 Phe Thr Pro Ile Ala Ala Asp Leu Gly Val Gly Pro Leu Ile Val Leu
 195 200 205
 Arg Ala Leu Glu Gly Leu Gly Glu Gly Val Thr Phe Pro Ala Met His
 210 215 220
 Ala Met Trp Ser Ser Trp Ala Pro Pro Leu Glu Arg Ser Lys Leu Leu
 225 230 235 240
 Ser Ile Ser Tyr Ala Gly Ala Gln Leu Gly Thr Val Ile Ser Leu Pro
 245 250 255
 Leu Ser Gly Ile Ile Cys Tyr Tyr Met Asn Trp Thr Tyr Val Phe Tyr
 260 265 270
 Phe Phe Gly Thr Ile Gly Ile Phe Trp Phe Leu Leu Trp Ile Trp Leu
 275 280 285
 Val Ser Asp Thr Pro Gln Lys His Lys Arg Ile Ser His Tyr Glu Lys
 290 295 300
 Glu Tyr Ile Leu Ser Ser Leu Arg Asn Gln Leu Ser Ser Gln Lys Ser
 305 310 315 320
 Val Pro Trp Val Pro Ile Leu Lys Ser Leu Pro Leu Trp Ala Ile Val
 325 330 335
 Val Ala His Phe Ser Tyr Asn Trp Thr Phe Tyr Thr Leu Leu Thr Leu
 340 345 350
 Leu Pro Thr Tyr Met Lys Glu Ile Leu Arg Phe Asn Val Gln Glu Asn
 355 360 365

Gly Phe Leu Ser Ser Leu Pro Tyr Leu Gly Ser Trp Leu Cys Met Ile
 370 375 380
 Leu Ser Gly Gln Ala Ala Asp Asn Leu Arg Ala Lys Trp Asn Phe Ser
 385 390 395 400
 Thr Leu Cys Val Arg Arg Ile Phe Ser Leu Ile Gly Met Ile Gly Pro
 405 410 415
 Ala Val Phe Leu Val Ala Ala Gly Phe Ile Gly Cys Asp Tyr Ser Leu
 420 425 430
 Ala Val Ala Phe Leu Thr Ile Ser Thr Thr Leu Gly Gly Phe Cys Ser
 435 440 445
 Ser Gly Phe Ser Ile Asn His Leu Asp Ile Ala Pro Ser Tyr Ala Gly
 450 455 460
 Ile Leu Leu Gly Ile Thr Asn Thr Phe Ala Thr Ile Pro Gly Met Val
 465 470 475 480
 Gly Pro Val Ile Ala Lys Ser Leu Thr Pro Asp Asn Thr Val Gly Glu
 485 490 495
 Trp Gln Thr Val Phe Tyr Ile Ala Ala Ala Ile Asn Val Phe Gly Ala
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 Asn Asp His His Gly His Arg His
 530 535

<210> 9

<211> 1485

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: human/sheep
consensus sequence

<220>

<221> CDS

<222> (1) .. (1485)

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Met	Xaa	Xaa	Pro	Val	Xaa	Asp	Xaa	Ala	Xaa	Xaa	Xaa	Gly	Glu	Glu	Xaa	
1				5				10					15			
wcg	gac	cgc	acr	ckk	cty	ctr	cmg	sgc	gcc	ccr	cgg	gcs	gaa	scc	gct	96
Xaa	Asp	Arg	Xaa	Xaa	Xaa	Xaa	Xaa	Ala	Xaa	Arg	Xaa	Glu	Xaa	Ala		
			20					25				30				
cca	gtr	tgc	tgc	tct	gct	cgt	tac	aac	yta	gca	wtt	ttg	kcc	ttt	ttt	144
Pro	Xaa	Cys	Cys	Ser	Ala	Arg	Tyr	Asn	Xaa	Ala	Xaa	Leu	Xaa	Phe	Phe	
		35				40						45				
ggt	ttc	ttc	rtt	sts	tat	kca	tta	cgk	gtg	aat	ctg	agy	gtt	gcr	yta	192
Gly	Phe	Phe	Xaa	Xaa	Tyr	Xaa	Leu	Xaa	Val	Asn	Leu	Xaa	Val	Xaa	Xaa	
	50					55					60					
gtg	gay	atg	gtr	gat	tca	aay	aca	act	kym	raa	gat	aat	aga	ack	tcc	240
Val	Xaa	Met	Xaa	Asp	Ser	Xaa	Thr	Thr	Xaa	Xaa	Asp	Asn	Arg	Xaa	Ser	
65					70					75				80		
was	gmg	tgt	sca	gag	cat	tct	gct	ccc	ata	aaa	gtt	cwt	cay	aay	caa	288
Xaa	Xaa	Cys	Xaa	Glu	His	Ser	Ala	Pro	Ile	Lys	Val	Xaa	Xaa	Xaa	Gln	
			85					90						95		
acg	ggt	aar	aag	tac	crr	tgg	gat	gca	gaa	act	caa	gga	tgg	att	ctc	336
Thr	Gly	Xaa	Lys	Tyr	Xaa	Trp	Asp	Ala	Glu	Thr	Gln	Gly	Trp	Ile	Leu	
			100					105					110			
ggw	tcy	ttt	tty	tat	ggc	tac	atc	atc	aca	car	att	cct	gga	gga	tat	384
Xaa	Xaa	Phe	Xaa	Tyr	Gly	Tyr	Ile	Ile	Thr	Xaa	Ile	Pro	Gly	Gly	Tyr	
		115					120					125				
gtt	gcc	agc	ara	akw	ggg	ggg	aar	mtg	ytg	cta	gga	tty	ggg	atc	ytt	432
Val	Ala	Ser	Xaa	Xaa	Gly	Gly	Xaa	Xaa	Xaa	Leu	Gly	Xaa	Gly	Ile	Xaa	
	130					135					140					
gsy	acw	gct	rtc	ytic	acc	ctg	ttc	act	ccc	mtg	gct	gca	gat	ttm	gga	480
Xaa	Xaa	Ala	Xaa	Xaa	Thr	Leu	Phe	Thr	Pro	Xaa	Ala	Ala	Asp	Xaa	Gly	
145					150					155				160		
gty	gga	scm	cty	rtt	gya	ctc	agr	gca	cta	gaa	ggr	cta	gga	gag	ggt	528
Xaa	Gly	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Ala	Leu	Glu	Xaa	Leu	Gly	Glu	Gly	
			165					170					175			
gty	aca	twt	cca	gcc	atg	cat	gcc	atg	tgg	tct	tcw	tgg	gct	ccc	cct	576
Xaa	Thr	Xaa	Pro	Ala	Met	His	Ala	Met	Trp	Ser	Xaa	Trp	Ala	Pro	Pro	
			180					185					190			

ctt gaa aga agc aar ctt ctk agy att tcr tat gca gga gca car ctt 624
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 195 200 205

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 Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met
 210 215 220

aat tgg act tat gtc ttc tay tty ttt ggy ayt rtt gga atm wty tgg 720
 Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp
 225 230 235 240

ttt mtt ttr tgg atc tgs tta gtt agt gay aca cca saa amw cac aag 768
 Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys
 245 250 255

asa aty wcy cmy tat gaa aag gar tay att ctt tca tca tta ara aat 816
 Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn
 260 265 270

cag cty tct tca cag aag tca gtg ccg tgg rta ccy atk ytr aaa tcm 864
 Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa
 275 280 285

ctg cca ctt tgg gct aty gtm gtt gca cay ttt tct tac aac tgg act 912
 Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr
 290 295 300

ttt tat act ttr ttg acm tta ttg cct act tay atg aag gar rtc cta 960
 Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu
 305 310 315 320

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 325 330 335

ggy tst tgg tta tgt atg atc ctg tck ggt caa gct gct gac aat tta 1056
 Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
 340 345 350

agg gca ara tgg aat ttt tca act ytr tgk gtt cgm aga rtt ttt agc 1104
 Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser
 355 360 365

ctt ata ggr atg att gga cct gcr rta ttc ctg gtw gcy gcw ggm tty 1152
 Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa
 370 375 380

atw ggc tgt gat tat tcy ttg gcy gtt gcw ttc cta acy ata tca aca 1200
 Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr
 385 390 395 400
 acm ctg gga ggc ttt tgc tct tct gga ttt agc atc aac cat ctg gay 1248
 Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa
 405 410 415
 att gct cct tcg tat gct ggt aty ctc ctg ggc atc aca aat acm ttt 1296
 Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe
 420 425 430
 gcc act att ccw gga atg rtt ggg ccc rtc att gcy ara agt ctk acc 1344
 Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr
 435 440 445
 cct gak aac act rtt gga gaa tgg caa acy gtc ttc try aty gct gct 1392
 Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala
 450 455 460
 gct aty aat gtw ttt ggt gcc att ttc tty aca cta ttc gcc aaa ggt 1440
 Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly
 465 470 475 480
 gaa gtr caa aac tgg gcy mtc art gat cac caw gga cac aga mac 1485
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 485 490 495

<210> 10
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 <212> PRT
 <213> Artificial Sequence

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 Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe
 35 40 45
 Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa
 50 55 60

Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser
 65 70 75 80
 Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln
 85 90 95
 Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
 100 105 110
 Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
 115 120 125
 Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa
 130 135 140
 Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
 145 150 155 160
 Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
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 Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro
 180 185 190
 Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu
 195 200 205
 Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met
 210 215 220
 Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp
 225 230 235 240
 Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys
 245 250 255
 Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn
 260 265 270
 Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa
 275 280 285
 Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr
 290 295 300
 Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu
 305 310 315 320

Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu
 325 330 335
 Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
 340 345 350
 Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser
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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/16676

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/705 G01N33/50 A61K38/17 //C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL - EMBEST20 'Online! Entry HS1173506, Acc.no. AA258513, 19 March 1997 (1997-03-19) HILLIER, L. ET AL.: "zr59d01.r1 Soares NhhMPu S1 Homo sapiens cDNA clone 667681 5' similar to TR:G507415 G507415 BRAIN SPECIFIC NA+-DEPENDENT INORGANIC PHOSPHATE COTRANSPORTER." XP002121520 the whole document</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1,4-6

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

5 November 1999

Date of mailing of the international search report

17/11/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax: (+31-70) 340-3016

Authorized officer

Smalt, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/16676

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HELLERQVIST C G ET AL: "ANTITUMOR EFFECTS OF GBS TOXIN: A POLYSACCHARIDE EXOTOXIN FROM GROUP B BETA-HEMOLYTIC STREPTOCOCCUS" JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY, vol. 120, no. 1/02, 1 January 1993 (1993-01-01), pages 63-70, XP000749401 ISSN: 0171-5216 the whole document ---	1-20,23, 25,26, 28-40
Y	GEARING, D.P. ET AL.: "Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor." EMBO JOURNAL, vol. 8, no. 12, 1989, pages 3667-76, XP002121518 abstract ---	1-20,23, 25,26, 28-40
P,X	FU, C. ET AL.: "Expressional cloning of CM101 receptor gene from mammalian cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION OF CANCER RESEARCH, vol. 40, March 1999 (1999-03), pages 557-Abstr.3677, XP002121519 the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/16676

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION SHEET PCT/ISA/210
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claims 24, 39, 40 and 44 could not be searched to completion due to insufficient characterization of the inhibitors in the description.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99 /16676

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 39, and 40 in as far as it pertains to an in vivo method, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claim 22, in as far as it relates to a method for use in vivo, is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims 24, 39, 40 and 44 could not be searched to completion due to insufficient characterization of the inhibitors in the description.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

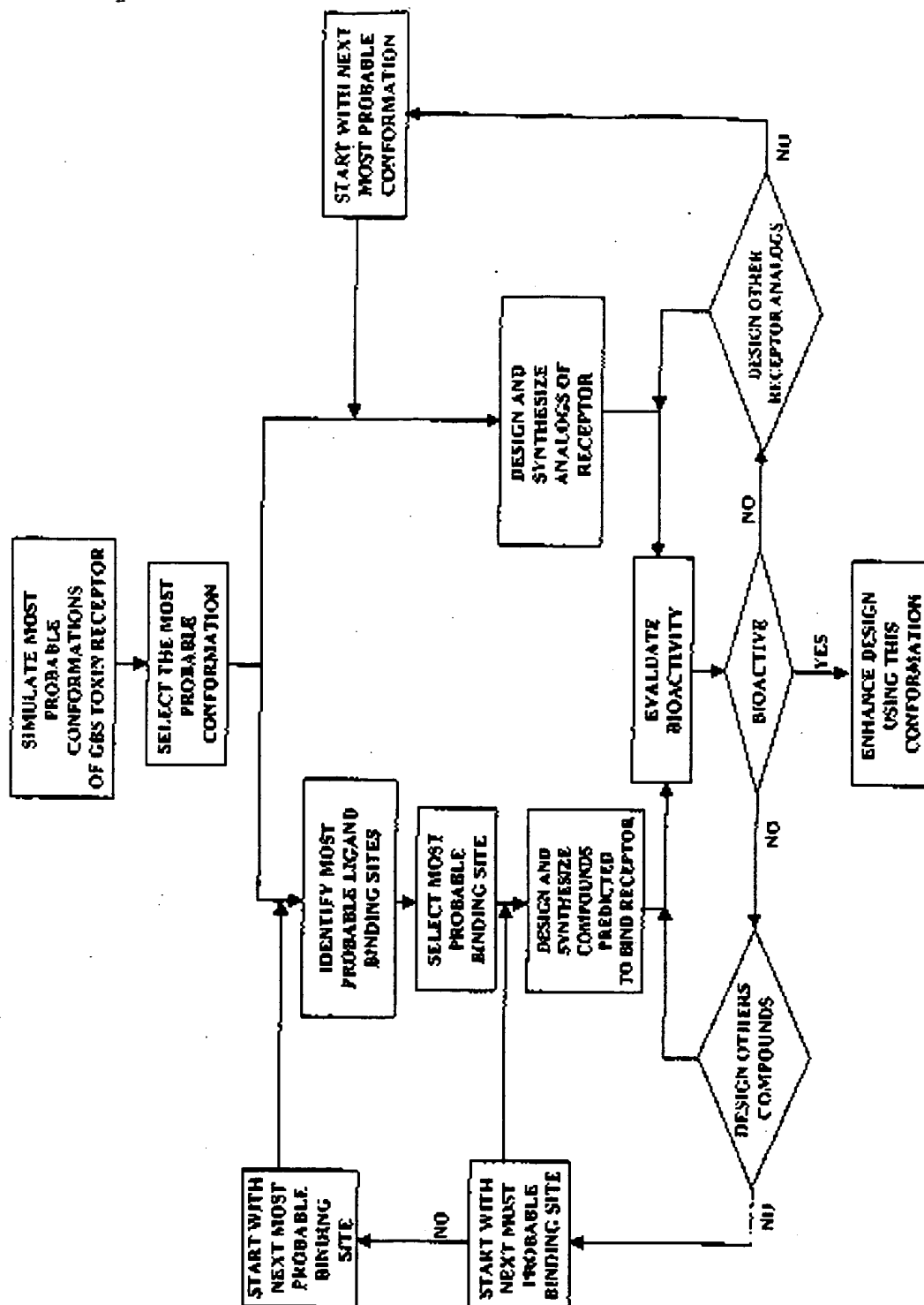


FIG. 1



**Normal Human
Ovary+Pab 55**

FIG. 2B



**Human Ovary
Cancer+Pab 55**

FIG. 2A



**Normal Human
Ovary+Pab 57**

FIG. 3B



**Human Ovary
Cancer+Pab 57**

FIG. 3A



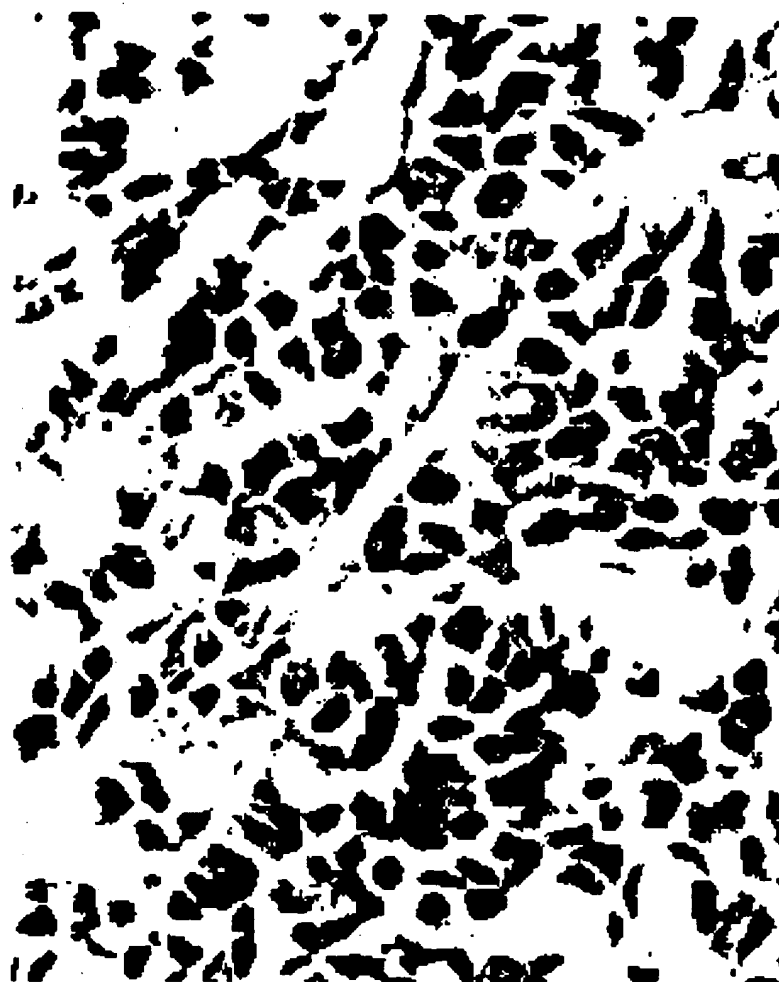
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+McAb**

FIG. 4B



**MLT CM101-Biot.5'
+Strep.HRP**

FIG. 4A



MLT-PBS 5' + Streptavidin-HRP

FIG. 4C

SEQUENCE LISTING

<110> Hellerqvist, Carl
Fu, Changlin

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 Gly Thr Val Ile Ser Leu Pro Leu Ser Gly Ile Ile Cys Tyr Tyr Met
 210 215 220

Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Thr Ile Gly Ile Phe Trp
 225 230 235 240
 Phe Leu Leu Trp Ile Trp Leu Val Ser Asp Thr Pro Gln Lys His Lys
 245 250 255
 Arg Ile Ser His Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Arg Asn
 260 265 270
 Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Val Pro Ile Leu Lys Ser
 275 280 285
 Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr
 290 295 300
 Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Ile Leu
 305 310 315 320
 Arg Phe Asn Val Gln Glu Asn Gly Phe Leu Ser Ser Leu Pro Tyr Leu
 325 330 335
 Gly Ser Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu
 340 345 350
 Arg Ala Lys Trp Asn Phe Ser Thr Leu Cys Val Arg Arg Ile Phe Ser
 355 360 365
 Leu Ile Gly Met Ile Gly Pro Ala Val Phe Leu Val Ala Ala Gly Phe
 370 375 380
 Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr
 385 390 395 400
 Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp
 405 410 415
 Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe
 420 425 430
 Ala Thr Ile Pro Gly Met Val Gly Pro Val Ile Ala Lys Ser Leu Thr
 435 440 445
 Pro Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala
 450 455 460
 Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
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<211> 2844

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<213> Ovis sp.

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 Met Lys Ser Pro Val Ser Asp Leu Ala Pro
 1 5 10

agc gac ggc gag gag ggc tcg gac cgc aca ccg ctc ctg cag cgc gcc 161
 Ser Asp Gly Glu Glu Gly Ser Asp Arg Thr Pro Leu Leu Gln Arg Ala
 15 20 25

ccg cgg gcg gaa ccc gct cca gta tgc tgc tct gct cgt tac aac cta 209
 Pro Arg Ala Glu Pro Ala Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu
 30 35 40

gca ttt tcg tcc tct ttc ggt ttc ttc gtt ctc tat tca tta cgg gtg 257
 Ala Phe Leu Ser Phe Phe Gly Phe Phe Val Leu Tyr Ser Leu Arg Val
 45 50 55

aat ctg agc gtt gca cta gtg gac atg gtg gat tca aac aca act gcc 305
 Asn Leu Ser Val Ala Leu Val Asp Met Val Asp Ser Asn Thr Thr Ala
 60 65 70

aaa gat aat aga acg tcc tac gag tgt gca gag cat tct gct ccc ata 353
 Lys Asp Asn Arg Thr Ser Tyr Glu Cys Ala Glu His Ser Ala Pro Ile
 75 80 85 90

aaa gtt ctt caa aac caa acg ggt aaa aag tac cgg tgg gat gca gaa 401
 Lys Val Leu His Asn Gln Thr Gly Lys Lys Tyr Arg Trp Asp Ala Glu
 95 100 105

act caa gga tgg att ctc gga tct ttt ttc tat ggc tac atc atc aca 449
 Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr
 110 115 120

caa att cct gga gga tat gtt gcc agc aga agt ggg ggg aag ctg ttg	497
Gln Ile Pro Gly Gly Tyr Val Ala Ser Arg Ser Gly Gly Lys Leu Leu	
125 130 135	
cta gga ttc ggg atc ttt gct aca gct acc ttc acc ctg ctg act ccc	545
Leu Gly Phe Gly Ile Phe Ala Thr Ala Ile Phe Thr Leu Phe Thr Pro	
140 145 150	
ctc gct gca gat ttc gga gtc gga gcc ctt gtt gca ctg agg gca cta	593
Leu Ala Ala Asp Phe Gly Val Gly Ala Leu Val Ala Leu Arg Ala Leu	
155 160 165 170	
gaa ggg cta gga gag ggt gtc aca tat cca gcc atg cat gcc atg tgg	641
Glu Gly Leu Gly Glu Gly Val Thr Tyr Pro Ala Met His Ala Met Trp	
175 180 185	
tct tca tgg gct ccc cct ctt gaa aga agc aag ctt ctg agt att tca	689
Ser Ser Trp Ala Pro Pro Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser	
190 195 200	
tat gca gga gca caa ctt ggg aca gta gtt tct ctt cct ctt tct gga	737
Tyr Ala Gly Ala Gln Leu Gly Thr Val Val Ser Leu Pro Leu Ser Gly	
205 210 215	
gta att tgc tac tat atg aat tgg act tat gtc ttc tat ttc ttt ggc	785
Val Ile Cys Tyr Tyr Met Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly	
220 225 230	
att gtt gga atc atc tgg ttt att tca tgg atc tgc tta gtt agt gat	833
Ile Val Gly Ile Ile Trp Phe Ile Leu Trp Ile Cys Leu Val Ser Asp	
235 240 245 250	
aca cca gaa act cac aag aca atc act ccc tat gaa aag gag tat att	881
Thr Pro Glu Thr His Lys Thr Ile Thr Pro Tyr Glu Lys Glu Tyr Ile	
255 260 265	
ctt tca tca tta aaa aat cag ctg tct tca cag aag tca gtg ccg tgg	929
Leu Ser Ser Leu Lys Asn Gln Leu Ser Ser Gln Lys Ser Val Pro Trp	
270 275 280	
ata cct atg ctg aaa tca ctg cca ctt tgg gct att gtc gtt gca cat	977
Ile Pro Met Leu Lys Ser Leu Pro Leu Trp Ala Ile Val Val Ala His	
285 290 295	
ttt tct tac aac tgg act ctt tat act ttg ttg acc tca ttg cct act	1025
Phe Ser Tyr Asn Trp Thr Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr	
300 305 310	

tac atg aag gaa gtc cta agg ttc aat att caa gag aat ggg ttt tta	1073
Tyr Met Lys Glu Val Leu Arg Phe Asn Ile Gln Glu Asn Gly Phe Leu	
315 320 325 330	
tct gca gtc cct tat tta ggt tgt tgg tta tgt atg atc ctg tgg ggt	1121
Ser Ala Val Pro Tyr Leu Gly Cys Trp Leu Cys Met Ile Leu Ser Gly	
335 340 345	
caa gct gct gac aat tta agg gca aga tgg aat ttt tca act ctg tgg	1169
Gln Ala Ala Asp Asn Leu Arg Ala Arg Trp Asn Phe Ser Thr Leu Trp	
350 355 360	
gtt cga aga gtt ttt agc ctt ata ggg atg att gga cct gcg ata ttc	1217
Val Arg Arg Val Phe Ser Leu Ile Gly Met Ile Gly Pro Ala Ile Phe	
365 370 375	
ctg gtt gcc gca gga ttt ata ggc tgt gat tat tcc ttg gct gtt gca	1265
Leu Val Ala Ala Gly Phe Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala	
380 385 390	
ttc cta acc ata tca aca acc ctg gga ggc ttt tgc tct tct gga ttt	1313
Phe Leu Thr Ile Ser Thr Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe	
395 400 405 410	
agc atc aac cat ctg gac att gct cct tgg tat gct ggt att ctc ctg	1361
Ser Ile Asn His Leu Asp Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu	
415 420 425	
ggc atc aca aat acc ttt gcc act att cct gga atg att ggg gcc atc	1409
Gly Ile Thr Asn Thr Phe Ala Thr Ile Pro Gly Met Ile Gly Pro Ile	
430 435 440	
att gcc aga agt ctt acc cct gag aac act att gga gaa tgg caa act	1457
Ile Ala Arg Ser Leu Thr Pro Glu Asn Thr Ile Gly Glu Trp Gln Thr	
445 450 455	
gtt ttc tgc atc gct gct gct atc aat gta ttt ggt gcc att ttc ttc	1505
Val Phe Cys Ile Ala Ala Ala Ile Asn Val Phe Gly Ala Ile Phe Phe	
460 465 470	
aca cta ttc gcc aaa ggt gaa gtg caa aac tgg gcc atc agt gat cac	1553
Thr Leu Phe Ala Lys Gly Glu Val Gln Asn Trp Ala Ile Ser Asp His	
475 480 485 490	
caa gga cac aga aac tgaagggaacc aataaataat cctgtctctta ttaattgtatc	1608
Gln Gly His Arg Asn	
495	

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 caagataaaa ttgtactaga aaaattgtgt tagatttgta aggccttgtaa tcatgaaacg 1728
 tcactagttg ccatataagc aaaattagct atttttaatt attattaacg cgtttgctgg 1789
 aacttacaat tcagggtcac atatctggct gcaagtcagg caaccacac taggggagtt 1848
 ctatttattt ataagacctt acctaaagag atgagctgaa atagacctt ctataacctt 1908
 gcttaattaa ggtggataat aattctcagg tcttgtaaa catctgtttt tgcacacctt 1968
 cctcaaaaaa ttatttgta tcagcaatcc ctgacatgta ggtctcaaac tttagcctct 2028
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 gagggcattt atgtgattta gaacttgatt aatgtttcac tgtgtatgtt caacactacc 2688
 tgtaatatzt taactaaagc tatttaagt atatatgagt gtatacatc tgcataattaa 2748
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 <212> PRT
 <213> Ovis sp.

<400> 4

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35 40 45

Gly Phe Phe Val Leu Tyr Ser Leu Arg Val Asn Leu Ser Val Ala Leu
50 55 60

Val Asp Met Val Asp Ser Asn Thr Thr Ala Lys Asp Asn Arg Thr Ser
65 70 75 80

Tyr Glu Cys Ala Glu His Ser Ala Pro Ile Lys Val Leu His Asn Gln
85 90 95

Thr Gly Lys Lys Tyr Arg Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
100 105 110

Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr
115 120 125

Val Ala Ser Arg Ser Gly Gly Lys Leu Leu Leu Gly Phe Gly Ile Phe
130 135 140

Ala Thr Ala Ile Phe Thr Leu Phe Thr Pro Leu Ala Ala Asp Phe Gly
145 150 155 160

Val Gly Ala Leu Val Ala Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly
165 170 175

Val Thr Tyr Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro
180 185 190

Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu
195 200 205

Gly Thr Val Val Ser Leu Pro Leu Ser Gly Val Ile Cys Tyr Tyr Met
210 215 220

Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Ile Val Gly Ile Ile Trp
225 230 235 240

Phe Ile Leu Trp Ile Cys Leu Val Ser Asp Thr Pro Glu Thr His Lys
245 250 255

Thr Ile Thr Pro Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Lys Asn
 260 265 270
 Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Ile Pro Met Leu Lys Ser
 275 280 285
 Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr
 290 295 300
 Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Val Leu
 305 310 315 320
 Arg Phe Asn Ile Gln Glu Asn Gly Phe Leu Ser Ala Val Pro Tyr Leu
 325 330 335
 Gly Cys Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu
 340 345 350
 Arg Ala Arg Trp Asn Phe Ser Thr Leu Trp Val Arg Arg Val Phe Ser
 355 360 365
 Leu Ile Gly Met Ile Gly Pro Ala Ile Phe Leu Val Ala Ala Gly Phe
 370 375 380
 Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr
 385 390 395 400
 Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp
 405 410 415
 Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe
 420 425 430
 Ala Thr Ile Pro Gly Met Ile Gly Pro Ile Ile Ala Arg Ser Leu Thr
 435 440 445
 Pro Glu Asn Thr Ile Gly Glu Trp Gln Thr Val Phe Cys Ile Ala Ala
 450 455 460
 Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
 465 470 475 480
 Glu Val Gln Asn Trp Ala Ile Ser Asp His Gln Gly His Arg Asn
 485 490 495

<210> 5

<211> 31
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 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: PCR primer

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31

<210> 6
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: PCR primer

<400> 6
 ggaattccdg gdgratktc narrrrrtt

29

<210> 7
 <211> 2930
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (263) .. (1870)

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 gggggagacg gtcttcgaa caccggetcc cggcatgag tagaccggcg gggggagcgg 180
 gctcactttg cgcctactct acgagaactc ccagaactcc gcttccctag tccaccccaa 240
 gccagagttg cccacacctc ag atg gcg gcg ggg gcg atg aca ccg ccc cgc 292
 Met Ala Ala Gly Ala Met Thr Pro Pro Arg
 1 5 10
 ccg gtc cag cca gct cgg ccc ggg ggc ttc ggg ctg tgg ggc cgg cgc 340
 Pro Val Gln Pro Ala Arg Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg
 15 20 25

tcc ctt ctc tgc cag gta gog agt aca cct gcc cac gta ggc gtc atg 388
 Ser Leu Leu Cys Gln Val Ala Ser Thr Pro Ala His Val Gly Val Met
 30 35 40

agg tct cgg gtt cga gac ctg gcc cgg aac gat ggc gag gag agc arg 436
 Arg Ser Pro Val Arg Asp Leu Ala Arg Asn Asp Gly Glu Glu Ser Thr
 45 50 55

gac cgc acg cct ctt cta ccg ggc gcc cca cgg gcc gaa gcc gct cca 484
 Asp Arg Thr Pro Leu Leu Pro Gly Ala Pro Arg Ala Glu Ala Ala Pro
 60 65 70

gtg tgc tgc tct gct cgt tac aac tta gca att ttg gcc ttt ttt ggt 532
 Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Ile Leu Ala Phe Phe Gly
 75 80 85 90

ttc ttc att gtg tat gca tta cgt gtg aat ctg agt gtt gcg tta gtg 580
 Phe Phe Ile Val Tyr Ala Leu Arg Val Asn Leu Ser Val Ala Leu Val
 95 100 105

gat atg gta gat tca aat aca acg tta gaa gat aat aga act tcc aag 628
 Asp Met Val Asp Ser Asn Thr Thr Leu Glu Asp Asn Arg Thr Ser Lys
 110 115 120

gcg tgt cca gag cat tct gct ccc ata aaa gtt cat cat aat caa acg 676
 Ala Cys Pro Glu His Ser Ala Pro Ile Lys Val His His Asn Gln Thr
 125 130 135

ggt aag aag tac caa tgg gat gca gaa act caa gga tgg att ctc ggt 724
 Gly Lys Lys Tyr Gln Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu Gly
 140 145 150

tcc tct ttt tat ggc tac atc atc aca cag att cct gga gga tat gtt 772
 Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr Val
 155 160 165 170

gcc agc aaa ata ggg ggg aaa atg ctg cta gga ttt ggg atc ctt ggc 820
 Ala Ser Lys Ile Gly Gly Lys Met Leu Leu Gly Phe Gly Ile Leu Gly
 175 180 185

act gcc gtc ctc acc ctg ttc act ccc att gct gca gat tta gga gtt 868
 Thr Ala Val Leu Thr Leu Phe Thr Pro Ile Ala Ala Asp Leu Gly Val
 190 195 200

gga cca ctc att gta ctc aga gca cta gaa gga cta gga gag ggt gtt 916
 Gly Pro Leu Ile Val Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly Val
 205 210 215

aca ttt cca gcc atg cat gcc atg tgg cct tct tgg gct ccc oct ctt 964
 Thr Phe Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro Leu
 220 225 230

gaa aga agc aaa ctt ctt agc att tgg tat gca gga gcc cag ctt ggg 1012
 Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu Gly
 235 240 245 250

aca gta att tct ctt cct ctt tct gga ata att cgc tac tat acg aac 1060
 Thr Val Ile Ser Leu Pro Leu Ser Gly Ile Ile Cys Tyr Tyr Met Asn
 255 260 265

tgg act cat gtc ttc tac ttt ttt ggt act att gga ata ttt tgg ttt 1108
 Trp Thr Tyr Val Phe Tyr Phe Phe Gly Thr Ile Gly Ile Phe Trp Phe
 270 275 280

ctt ttg tgg atc tgg tta gtt agt gac aca cca caa aaa cac aag aga 1156
 Leu Leu Trp Ile Trp Leu Val Ser Asp Thr Pro Gln Lys His Lys Arg
 285 290 295

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 Ile Ser His Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Arg Asn Gln
 300 305 310

ctt tct tca cag aag tca gtg cgg tgg gta ccc att tta aaa tcc ctg 1252
 Leu Ser Ser Gln Lys Ser Val Pro Trp Val Pro Ile Leu Lys Ser Leu
 315 320 325 330

cca ctt tgg gct atc gta gtt gca cac ttt tct tac aac tgg act ttt 1300
 Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr Phe
 335 340 345

tat act tta ttg aca tta ttg cct act tat atg aag gag atc cta agg 1348
 Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Ile Leu Arg
 350 355 360

ttc aat gtt caa gag aat ggg ttt tta tct tca ttg cct tat tta ggc 1396
 Phe Asn Val Gln Glu Asn Gly Phe Leu Ser Ser Leu Pro Tyr Leu Gly
 365 370 375

tct tgg tta tgt atg atc ctg tct ggt caa gct gct gac aat tta agg 1444
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 380 385 390

gca aaa tgg aat ttt tca act tta tgt gtt cgc aga att ttt agc ctt 1492
 Ala Lys Trp Asn Phe Ser Thr Leu Cys Val Arg Arg Ile Phe Ser Leu
 395 400 405 410

ata gga atg att gga cct goa gta ttc ctg gta gcc gct ggc ttc act 1540
 Ile Gly Met Ile Gly Pro Ala Val Phe Leu Val Ala Ala Gly Phe Ile
 415 420 425

ggc tgt gat tat tct ttg gcc gtt gct ttc cta act ata tca aca aca 1568
 Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr Thr
 430 435 440

ctg gga ggc ttt tgc tct tct gga ttt agc atc aac cat ctg gat att 1636
 Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp Ile
 445 450 455

gct cct tgg tat gct ggt atc ctc ctg ggc atc aca aat aca ttt gcc 1684
 Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe Ala
 460 465 470

act att cca gga atg gtt ggg ccc gtc att gct aaa agt ctg acc cct 1732
 Thr Ile Pro Gly Met Val Gly Pro Val Ile Ala Lys Ser Leu Thr Pro
 475 480 485 490

gat aac act gtt gga gaa tgg caa acc gtg ttc tat att gct gct gct 1780
 Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala Ala
 495 500 505

att aat gtt ttt ggt gcc att ttc ttc aca cta ttc gcc aaa ggt gaa 1828
 Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly Glu
 510 515 520

gta caa aac tgg gct ctc aat gat cac cat gga cac aga cac 1870
 Val Gln Asn Trp Ala Leu Asn Asp His His Gly His Arg His
 525 530 535

tgaaggaacc aataataat cctgcctcta ttaattgtatt tttattttatc atgtaacctc 1930

aaagtgcctt ctgtattgtg taagcattct atgtcttttc ttaattgtac ttgtattaga 1990

tttttaagge ctataatcat gaaatatcac tagttgccag aataataaaa tgaactgtgt 2050

ttaattatga ataataatga agctaggact tctactttag gtacacatca ctgcttgeta 2110

gtcgggcaac atgaagttag acagttctgt tgatttttta gggccatact aaaggggaatg 2170

agctgaasca gacctctga tacctttgct taattaaact agatgatsat tctcaggtac 2230

tgataaacac ctgttgttgt tcactttctc cataaaaaatc gtcagctctc tctgacactc 2290

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<211> 536

<212> PRT

<213> Homo sapiens

<400> 8

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			20					25					30		
Ala	Ser	Thr	Pro	Ala	His	Val	Gly	Val	Met	Arg	Ser	Pro	Val	Arg	Asp
		35					40					45			
Leu	Ala	Arg	Asn	Asp	Gly	Glu	Glu	Ser	Thr	Asp	Arg	Thr	Pro	Leu	Leu
	50					55					60				
Pro	Gly	Ala	Pro	Arg	Ala	Glu	Ala	Ala	Pro	Val	Cys	Cys	Ser	Ala	Arg
	65				70					75				80	
Tyr	Asn	Leu	Ala	Ile	Leu	Ala	Phe	Phe	Gly	Phe	Phe	Ile	Val	Tyr	Ala
			85						90					95	
Leu	Arg	Val	Asn	Leu	Ser	Val	Ala	Leu	Val	Asp	Met	Val	Asp	Ser	Asn
		100						105					110		

Thr Thr Leu Glu Asp Asn Arg Thr Ser Lys Ala Cys Pro Glu His Ser
 115 120 125
 Ala Pro Ile Lys Val His His Asn Gln Thr Gly Lys Lys Tyr Gln Trp
 130 135 140
 Asp Ala Glu Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr
 145 150 155 160
 Ile Ile Thr Gln Ile Pro Gly Gly Tyr Val Ala Ser Lys Ile Gly Gly
 165 170 175
 Lys Met Leu Leu Gly Phe Gly Ile Leu Gly Thr Ala Val Leu Thr Leu
 180 185 190
 Phe Thr Pro Ile Ala Ala Asp Leu Gly Val Gly Pro Leu Ile Val Leu
 195 200 205
 Arg Ala Leu Glu Gly Leu Gly Glu Gly Val Thr Phe Pro Ala Met His
 210 215 220
 Ala Met Trp Ser Ser Trp Ala Pro Pro Leu Glu Arg Ser Lys Leu Leu
 225 230 235 240
 Ser Ile Ser Tyr Ala Gly Ala Gln Leu Gly Thr Val Ile Ser Leu Pro
 245 250 255
 Leu Ser Gly Ile Ile Cys Tyr Tyr Met Asn Trp Thr Tyr Val Phe Tyr
 260 265 270
 Phe Phe Gly Thr Ile Gly Ile Phe Trp Phe Leu Leu Trp Ile Trp Leu
 275 280 285
 Val Ser Asp Thr Pro Gln Lys His Lys Arg Ile Ser His Tyr Glu Lys
 290 295 300
 Glu Tyr Ile Leu Ser Ser Leu Arg Asn Gln Leu Ser Ser Gln Lys Ser
 305 310 315 320
 Val Pro Trp Val Pro Ile Leu Lys Ser Leu Pro Leu Trp Ala Ile Val
 325 330 335
 Val Ala His Phe Ser Tyr Asn Trp Thr Phe Tyr Thr Leu Leu Thr Leu
 340 345 350
 Leu Pro Thr Tyr Met Lys Glu Ile Leu Arg Phe Asn Val Gln Glu Asn
 355 360 365

Gly Phe Leu Ser Ser Leu Pro Tyr Leu Gly Ser Trp Leu Cys Met Ile
 370 375 380
 Leu Ser Gly Gln Ala Ala Asp Asn Leu Arg Ala Lys Trp Asn Phe Ser
 385 390 395 400
 Thr Leu Cys Val Arg Arg Ile Phe Ser Leu Ile Gly Met Ile Gly Pro
 405 410 415
 Ala Val Phe Leu Val Ala Ala Gly Phe Ile Gly Cys Asp Tyr Ser Leu
 420 425 430
 Ala Val Ala Phe Leu Thr Ile Ser Thr Thr Leu Gly Gly Phe Cys Ser
 435 440 445
 Ser Gly Phe Ser Ile Asn His Leu Asp Ile Ala Pro Ser Tyr Ala Gly
 450 455 460
 Ile Leu Leu Gly Ile Thr Asn Thr Phe Ala Thr Ile Pro Gly Met Val
 465 470 475 480
 Gly Pro Val Ile Ala Lys Ser Leu Thr Pro Asp Asn Thr Val Gly Glu
 485 490 495
 Trp Gln Thr Val Phe Tyr Ile Ala Ala Ala Ile Asn Val Phe Gly Ala
 500 505 510
 Ile Phe Phe Thr Leu Phe Ala Lys Gly Glu Val Gln Asn Trp Ala Leu
 515 520 525
 Asn Asp His His Gly His Arg His
 530 535

<210> 9

<211> 1485

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: human/sheep
consensus sequence

<220>

<221> CDS

<222> {1}..(1485)

<400> 9

atg arg tcy ccg gtt ysr gac ytr gcc csg arc gay ggc gag gag rgc 48
 Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa
 1 5 10 15

wcg gac cgc acr cck cty ctr cmg ege gcc ccr cgg gca gaa scc gct 96
 Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala
 20 25 30

cca gtr tgc tgc tct gct cgt tac aac yta gca wtr ttg kcc ttt ttt 144
 Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe
 35 40 45

ggc ttc ttc rtt etc tat kca tta cgg gtg aat ctg agy gct gcr yta 192
 Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa
 50 55 60

gtg gay atg gtr gat tca aay aca act kym raa gat aat aga ack tcc 240
 Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser
 65 70 75 80

was gmg tgt sca gag cat tct gct ccc ata aaa gtt cwt cay aay caa 288
 Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln
 85 90 95

acg ggt aar aag tac crr tgg gat gca gaa act caa gga tgg att etc 336
 Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
 100 105 110

ggw tcy ttt tty tat ggc tac atc atc aca car att cct gga gga tat 384
 Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
 115 120 125

gtt gcc agc ara akw ggg ggg ear mtg ytg cta gga tty ggg atc ytt 432
 Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa
 130 135 140

gay acw get rto ytc acc ctg ttc act ccc mty get gca gat ttm gga 480
 Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
 145 150 155 160

gty gga scm cty rtt gya rtc agr gca cta gaa ggr cta gga gag ggt 528
 Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
 165 170 175

gty aca twt cca gcc atg cat gcc atg tgg tct tcw egg gct ccc cct 576
 Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xa Trp Ala Pro Pro
 180 185 190

ctt gaa aga agc aar ctt ctk agy att tcr tat gca gga gca car ctt 624
 Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu
 195 200 205

ggg aca gta rtt tct ctt cct ctt tct gga rta att tgc tac tat atg 672
 Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met
 210 215 220

aat tgg act tat gtc ttc tay tty ttt ggy ayt rtt gga atm wty tgg 720
 Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp
 225 230 235 240

ttt mtt ttr tgg atc tgs tta gtt agt gay aca cca aaa amw cac aag 768
 Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys
 245 250 255

aaa aty wcy cmy tat gaa aag gar tay att ctt tca tca tta ara aat 816
 Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn
 260 265 270

cag cty tet tca cag aag tca gtg cgg tgg rta ccy atk ytr aaa tcm 864
 Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa
 275 280 285

ctg cca ctt tgg gct aty gtm gtt gca cay ttt tct tae aac tgg act 912
 Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr
 290 295 300

ttt tat act ttr tlg acm tta ttg cct act tay atg aag gar rto ota 960
 Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu
 305 310 315 320

agg ttc aat rtt caa gag aat ggg ttt tta tct kca kta cct tat tta 1008
 Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu
 325 330 335

ggy tct tgg tta tgt atg atc ctg tck ggt caa gct gct gac aat tta 1056
 Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
 340 345 350

agg gca ara tgg aat ttt tca act ytr tgg gtt cgm aga rtt ttt agc 1104
 Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser
 355 360 365

ctt ata ggr atg att gga cct gcr rta ttc ctg gtw gcy gcw ggm tty 1152
 Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa X a
 370 375 380

atw ggc tgc gat tat tcy ttg goy gtt gcw ttc cta acy ata tca aca 1200
 Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr
 385 390 395 400

acm ctg gga ggc ttt tgc tct tct gga ttt agc atc aac cat ctg gay 1248
 Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa
 405 410 415

att get cct tgc tat get ggt aty ctc ctg ggc atc aca aac acm ttt 1296
 Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe
 420 425 430

gcc act att ccw gga atg rtt ggg ccc ttc att goy ara agt ctk acc 1344
 Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr
 435 440 445

cct gak aac act rtt gga gaa tgg caa acy gtk ttc try aty got gct 1392
 Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala
 450 455 460

get aty aat gtw ttc ggt gcc att ttc tty aca cta ttc gcc aaa ggt 1440
 Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly
 465 470 475 480

gaa gtr caa aac tgg goy mtc art gat cac ccw gga cac aga mac 1485
 Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa
 485 490 495

<210> 10

<211> 495

<212> PRT

<213> Artificial Sequence

<400> 10

Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa
 2 5 10 15

Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala
 20 25 30

Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe
 35 40 45

Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa
 50 55 60

Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser
 65 70 75 80
 Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln
 85 90 95
 Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
 100 105 110
 Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
 115 120 125
 Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa
 130 135 140
 Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
 145 150 155 160
 Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
 165 170 175
 Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro
 180 185 190
 Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu
 195 200 205
 Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met
 210 215 220
 Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp
 225 230 235 240
 Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys
 245 250 255
 Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn
 260 265 270
 Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa
 275 280 285
 Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr
 290 295 300
 Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu
 305 310 315 320

Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu
 325 330 335
 Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
 340 345 350
 Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser
 355 360 365
 Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa
 370 375 380
 Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr
 385 390 395 400
 Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa
 405 410 415
 Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe
 420 425 430
 Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr
 435 440 445
 Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala
 450 455 460
 Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly
 465 470 475 480
 Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa
 485 490 495

<210> 11

<211> 1485

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: human/sheep
 consensus sequence

<220>

<221> CDS

<222> (1) .. (1485)

<400> 11

atg ang tcn ccg gtt nnn gac ntn gcc cng anc gan ggc gag gag ngo 48
 Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa
 1 5 10 15

ncg gac cgc acn tcn etn etn cng ngo gcc con cgg gcn gaa ncc gct 96
 Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala
 20 25 30

cca gtn tgc tgc tct gct cgt tac aac nta gca ntt ttg ncc ttc ttt 144
 Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe
 35 40 45

ggt ttc ttc ntt ntn tat nca tta cgn gtg aat ctg agn gtt gcn nta 192
 Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa
 50 55 60

gtg gan atg gtn gat tca aan aca act nnn naa gat aat aga acn tcc 240
 Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser
 65 70 75 80

nan gng tgt nca gag cat tct gct ccc ata aaa gtt cnt can aan caa 288
 Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Glu
 85 90 95

acg ggt aan aag tac cun tgg gat gca gaa act caa gga tgg att etc 336
 Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
 100 105 110

ggn tcn ttc ttn tat ggc tac atc atc aca can att cct gga gga tat 384
 Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
 115 120 125

gtt gcc agc ana ann ggg ggg aan ntg ntg cta gga ttn ggg atc ntt 432
 Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa
 130 135 140

gun acn gct ntc ntc acc ctg ttc act ccc ntn gct gca gat ttn gga 480
 Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
 145 150 155 160

gtn gga ncn otn ntt gna etc agn gca cta gaa ggn cta gga gag ggt 528
 Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
 165 170 175

gtn aca cnt cca gcc atg cat gcc atg tgg tct tcn tgg got ccc cct 576
 Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro
 180 185 190

ctt gaa aga agc aan ctt ctg agn att tcn tat gca gga gca can ctt Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu 195 200 205	624
ggg aca gta ntt tct ctt cct ctt tct gga nta att tgc tac tat atg Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met 210 215 220	672
aat tgg act tat gtc ttc tan ttn ttt ggn ant ntt gga atn ntn tgg Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp 225 230 235 240	720
ctt ntt ttn tgg atc tgn tta gtt agt gan aca cca naa ann cac aag Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys 245 250 255	768
ana atn ncn cnn tat gaa aag gan tan att ctt tca tca tta ana aat Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn 260 265 270	816
cag ctg tct tca cag aag tca gtc cgg tgg nta ccn atn ntn aaa tcn Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa 275 280 285	864
ctg cca ctt tgg gct atn gtn gtt gca can ttt tct tac aac tgg act Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr 290 295 300	912
ttt tat act ttn ttg acn tta ttg cct act tan atg aag gan ntc cta Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu 305 310 315 320	960
agg ttc aat ntt caa gag aat ggg ttt tta tct nca ntn cct tat tta Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu 325 330 335	1008
ggg tat tgg tta tgt atg atc ctg tcn ggt caa gct gct gac aat tta Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu 340 345 350	1056
agg gca ana tgg aat ttt taa act ntn tgn gtt ogn aga ntt ttt agc Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser 355 360 365	1104
ctt ata ggn atg att gga cct gcn nta ttc ctg gtn gcn gcn ggn ttn Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa 370 375 380	1152

atn ggc tgc gat tat tcn tgc gcn gtt gcn ttc cta acn ata tca aca 1200
 Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr
 385 390 395 400

 acn etg gga ggc ttt tgc tet tct gga ttt agc atc aac cat ctg gan 1248
 Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa
 405 410 415

 att gct cct tgc tat gct ggt atn ctc ctg ggc atc aca aat acn tct 1296
 Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe
 420 425 430

 gcc act att ccn gga atg ntt ggg ccc ntc att gcn ana agt ctn acc 1344
 Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr
 435 440 445

 cct gan aac act ntt gga gaa tgg caa acn gtn ttc tnn atn gct gct 1392
 Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala
 450 455 460

 gct atn aat gtn ttt ggt gcc att ttc ttn aca cta ttc gcc aaa ggt 1440
 Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly
 465 470 475 480

 gaa gtn caa aac tgg gcn ntc ant gat cac can gga cac aga nac 1485
 Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa
 485 490 495

<210> 12
 <211> 495
 <212> PRT
 <213> Artificial Sequence

<400> 12
 Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa
 1 3 10 15

 Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala
 20 25 30

 Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe
 35 40 45

 Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa
 50 55 60

 Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser

65	70	75	80
Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln	85	90	95
Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu	100	105	110
Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr	115	120	125
Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa	130	135	140
Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly	145	150	155
Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly	165	170	175
Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro	180	185	190
Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu	195	200	205
Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met	210	215	220
Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp	225	230	235
Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys	245	250	255
Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn	260	265	270
Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa	275	280	285
Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr	290	295	300
Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu	305	310	315
Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu			

325	330	335
Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu		
340	345	350
Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser		
355	360	365
Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa		
370	375	380
Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr		
385	390	395
Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa		
405	410	415
Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe		
420	425	430
Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr		
435	440	445
Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala		
450	455	460
Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly		
465	470	475
Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa		
485	490	495